View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Kentucky

University of Kentucky
UKnowledge

Theses and Dissertations--Animal and Food Sciences

Animal and Food Sciences

2018

INFLUENCE OF DIETARY RACTOPAMINE AND SUPRANUTRITIONAL SUPPLEMENTATION OF VITAMIN E ON PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM MUSCLE

Hyun Mok Kim
University of Kentucky, hmkim2002@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0002-5617-8356
Digital Object Identifier: https://doi.org/10.13023/ETD.2018.007

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Kim, Hyun Mok, "INFLUENCE OF DIETARY RACTOPAMINE AND SUPRANUTRITIONAL SUPPLEMENTATION OF VITAMIN E ON PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM MUSCLE" (2018). *Theses and Dissertations--Animal and Food Sciences*. 82.
https://uknowledge.uky.edu/animalsci_etds/82

This Master's Thesis is brought to you for free and open access by the Animal and Food Sciences at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Animal and Food Sciences by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Hyun Mok Kim, Student

Dr. Surendranath P. Suman, Major Professor

Dr. David L. Harmon, Director of Graduate Studies

INFLUENCE OF DIETARY RACTOPAMINE AND SUPRANUTRITIONAL SUPPLEMENTATION OF VITAMIN E ON PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM MUSCLE

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

Hyun Mok Kim
Lexington, Kentucky

Director: Dr. Surendranath P. Suman, Professor of Animal and Food Sciences
Lexington, Kentucky

2018

Copyright © Hyun Mok Kim 2018

ABSTRACT OF THESIS

INFLUENCE OF DIETARY RACTOPAMINE AND SUPRANUTRITIONAL SUPPLEMENTATION OF VITAMIN E ON PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM MUSCLE

The effects of dietary ingredients on the proteome profile of postmortem beef longissimus lumborum (LL) muscle were evaluated. In the first experiment, the influence of dietary ractopamine on the whole-muscle proteome of beef LL was examined. Five proteins were differentially abundant between ractopamine-fed (RAC) and non-ractopamine fed (CON) groups. The differentially abundant proteins were over-abundant in RAC and were related to muscle structure development (F-actin-capping protein subunit beta-2 and PDZ and LIM domain protein-3), chaperone (heat shock protein beta-1), oxygen transportation (myoglobin), and glycolysis (L-lactate dehydrogenase A chain). These findings indicated that ractopamine influences the abundance of proteins associated with muscle structure and fiber type shift in beef LL.

In the second experiment, the effect of Vitamin E supplementation on the sarcoplasmic proteome of beef LL was characterized. Five differentially abundant proteins were observed between vitamin E-supplemented (VITE) and non-vitamin E-supplemented (CONT) groups. All the differentially proteins were over-abundant in CONT and were associated with antioxidant activity (thioredoxin-dependent peroxide reductase, peroxiredoxin-6, and serum albumin) and glycolysis (beta-enolase and triosephosphate isomerase). These results indicated that the strong antioxidant activity of vitamin E leads to low expression of antioxidant proteins and antioxidant-related glycolytic enzymes in beef LL muscle.

KEYWORDS: ractopamine, vitamin E, proteomics, beef, longissimus lumborum

Hyun Mok Kim
Student's signature

01/04/2018
Date

INFLUENCE OF DIETARY RACTOPAMINE AND SUPRANUTRITIONAL SUPPLEMENTATION OF VITAMIN E ON PROTEOME PROFILE OF POSTMORTEM BEEF LONGISSIMUS LUMBORUM MUSCLE

By

Hyun Mok Kim

Dr. Surendranath P. Suman
Director of Thesis

Dr. David L. Harmon
Director of Graduate Studies

01/04/2018
Date

ACKNOWLEDGMENTS

Firstly, I would like to express my sincere gratitude to my major professor Dr. Surendranath P. Suman. Thank you for the strong support so that I have received the opportunity to pursue Master's degree at the University of Kentucky. I am always thankful to your careful devotion with patience. Without your advice and support, I would definitely have faced difficulties in succeeding in the program. I also would like to express my high appreciation to the committee members, Dr. Gregg Rentfrow and Dr. Rachel Schendel, for their help and support.

I sincerely thank Mahesh Nair and Shuting Li for helping me settle down in the lab. Thanks so much to Dr. Dustin Boler, University of Illinois, for providing the beef samples for my research projects. A special thanks to Dr. Carol Beach, University of Kentucky Proteomics Core Facility, for her assistance with mass spectrometric analyses. I would also appreciate the support received from Bruno Costa-Lima, Anna Canto, Chaoyu Zhai, Ana Paula, Prita Ashilola, and Yifei Wang in the lab.

Lastly, I would like to express my sincere thanks to my parents and family in South Korea for supporting me during the study. Also, thanks much to my brother and his wife, who live in Wisconsin, for their constant encouragement. Thanks to all my friends for their help and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Review of Literature

1.1. Feed additives in meat production

Meat is one of the primary sources for high quality animal protein in the world, and the production and consumption of meat is continuously increasing. To achieve the demands of increasing meat production, various strategies have been applied to improve growth efficiency in meat animals. For example, genetic and nutrition technologies are employed in the forms of animal breeding and improving the feed efficiency. Specifically, feed efficiency is a major factor in livestock production because it is strongly related to dietary nutrients and overall production cost (Patience et al., 2015).

Feeding a variety of animal feeds (or their combinations) has limitations to improve the quantity and quality of meat. Therefore, feed additives were developed to complement the feeding strategy. For instance, the emergence of metabolic modifiers helps to increase muscle deposition and decrease fat deposition, which increases production efficiency for farms, processing efficiency for meat packing plants, and economic efficiency for customers by enabling them to purchase meat at a less expensive price (Dunshea et al., 2005). Another example is vitamin E, which prevents lipid oxidation and increases meat color stability, and thus improves shelf life and marketability (Faustman et al., 1998).

1.2. Type of metabolic modifiers

1.2.1. Anabolic steroids

Anabolic steroids are approved for use in beef production, and steroid implants are approved in the U.S. since 1956 by FDA (Preston, 1999). In general, anabolic steroids exert significant effects on both heifers and steers; in general, while

estrogenic steroids are effective in steers, androgenic steroids are effective in heifers. However, these steroids are less effective in bulls (Dikeman, 2007) and swine (Hancock et al., 1991). The main effect of anabolic steroids is increased growth rate. Preston (1999) reviewed that steroid implants improve growth rate by 10-30%, feed efficiency by 5-15%, and carcass leanness by 5-8%. Furthermore, while implants increased feed intake by 5-10%, they increased feed efficiency by 5-15% (Dunshea et al., 2016). However, abuse of anabolic steroids may negatively affect meat quality. Morgan (1997) analyzed previous research data related to using anabolic steroids and found that excessive use of implant may decrease meat quality (marbling score and tenderness) and increase the incidence of dark-cutting. Furthermore, Duckett and Pratt (2014) suggested that timing of implant administration is critical to minimize the detrimental impact on marbling score and that the use of two or more implants in the finishing period may decrease tenderness.

1.2.2. Beta-adrenergic agonists

There are two beta-adrenergic agonists approved for use in the U.S. Ractopamine hydrochloride was approved in 2000 (FDA, 2000), and zilpaterol hydrochloride was approved in 2006 (FDA, 2006). Beta agonists functions as repartitioning agents, which stimulate skeletal muscles to grow and take away nutrients from adipose tissue (Beermann, 2002; Anderson et al., 2004; Almeida et al., 2012). Numerous studies indicated that beta agonists effectively increase lean muscle growth and minimize fat deposition (Bohrer et al., 2013; Arp et al., 2014; Edenburn et al., 2016) and increase feed efficiency (Rathmann et al., 2009). However, the ability of beta agonists to increase muscle deposition and decrease lipid

deposition may decrease tenderness and marbling (Arp et al., 2014; Lean et al., 2014). Moreover, recent research suggested that the incidence of death was 75-90% higher in beta agonists-fed cattle compared to the controls (Loneragan et al., 2014).

1.2.3. Immunocastration

Surgical castration has been traditionally utilized to increase meat quality, eliminate male pheromones, and reduce aggressive behavior in food animals. However, surgical castration may have negative effects, which includes the reduction of growth performance and excessive deposition of fat (Dunshea et al., 1993). Furthermore, modern-day meat consumers have negative perception of surgical castration because it is painful to the animals (Batorek et al., 2012). Therefore, as an alternative technology, immunocastration has been developed. Immunocastration is accomplished by immunization against gonadotropin-releasing hormone (GnRH), ultimately leading to inhibition of sex hormone production and effects similar to surgical castration. Gonadotropin-releasing hormone (GnRH) is a key hormone controlling sexual development and growth in animals.

Extensive research documented the beneficial effects of immunocastration, which improves carcass quality, reduces aggressive behavior, and reduces pheromones in cattle and pig (Huxsoll et al., 1998; Cook et al., 2000; Dunshea et al., 2001; Aïssat et al., 2002; Jaros et al., 2005; Janett et al., 2012; Dunshea et al., 2013). Moreover, meta-analysis by Batorek et al. (2012) on immunocastration in pigs concluded that immunocastrated pigs exhibited higher feed efficiency than intact male and surgically castrated pigs. In addition, immunocastrated animals had greater intramuscular fat content, and meat from these animals demonstrated lower shear

force compared to meat from intact males. Shi et al. (2016) analyzed the proteome of longissimus dorsi from immunocastrated and surgically castrated male pigs and found that the proteins associated in cytoskeletal, immunoglobulin, and metabolic enzymes were over-abundant in immunocastrated animals, whereas heat shock proteins and laminins are over-abundant in surgically castrated pigs possibly due to surgical stress. Furthermore, anti-GnRH vaccine does not interfere with other metabolic modifiers. For example, dietary ractopamine increased lean muscle in immunocastrated pigs and maximized the benefits of immunocastration (Rikard-Bell et al., 2009; Lanferdini et al., 2013). Furthermore, Amatayakul-Chantler et al. (2012) used implants in immunocastrated steers and found that the combination of implant and immunocastration increased body weight, hot carcass weight, and average daily gain more than either immunocastration or implant alone.

1.2.4. Vitamins

In the present day animal agriculture, vitamin E (α-tocopherol) is the most widely used vitamin as feed additive (Faustman et al., 1998). Supplementation of other vitamins may negatively affect meat quality. For example, vitamin D3 supplementation may reduce feed intake, decrease growth performance, and lead to potential toxicity in cattle, whereas vitamin A supplementation may have negative effects on marbling (Dikeman, 2007). Vitamin E is a lipid-soluble antioxidant that inhibits peroxidation of polyunsaturated fatty acids in cell membranes (Buttriss and Diplock, 1988). The α-tocopherol is the most chemically and biologically active form of vitamin E (Carr et al., 2000). Supplementation of vitamin E to meat animals minimizes oxidation of lipids and myoglobin in fresh meat (Dunshea et al., 2005). By

minimizing the oxidation of unsaturated fatty acids, α-tocopherol limits the formation of secondary byproducts of lipid oxidation such as carbonyls, which can bind with and oxidize myoglobin (Faustman et al., 2010). Vitamin E supplementation thus results in improved meat color stability (Faustman et al., 1998).

1.3. Specific feed additives

1.3.1. Ractopamine

1.3.1.1. Structure of ractopamine and distribution of beta-adrenergic agonist receptor (βAR)

Ractopamine is a phenylethanolamine derivative with beta-adrenergic agonist properties similar to the natural compound epinephrine and norepinephrine (Almeida et al., 2012). Ractopamine is made up of a phenolic ring substituted with an ethanolamine derivative, which carries a bulky butyl-phenol substituent on the aliphatic nitrogen (Smith, 1998). The structure contains two chiral centers and therefore exists as a mixture of four stereoisomers (RR, RS, SR, and SS) because two conformations can exist at each chiral carbon (Ricke et al., 1999).

There are three subtypes of β-adrenergic agonist receptors (βAR) in the mammalian cell membrane: β1AR, β2AR, and β3AR. Although some species have similar distribution of βAR subtypes, distribution differs based on species, tissues, and animal age (Mills et al., 2003). For examples, β1AR is mostly abundant in porcine adipose tissue, whereas β3AR is predominant in rat adipose tissue (McNeel and Mersmann, 1999). Furthermore, porcine liver βAR exhibits distribution of β1AR (45%) and β2AR (55%) subtypes (McNeel and Mersmann, 1999), whereas distribution of β2AR was dominant in cattle liver (β2AR > β1AR > β3AR), and β2AR increased with

age (Carron et al., 2005).

1.3.1.2. Beta-adrenergic receptor signaling pathway

The initiation of βAR signaling pathway starts when ractopamine binds to βAR, leading to activation of guanine nucleotide binding protein (G protein). G protein, consisting of Gα, Gβ, and Gγ subunits, is coupled with guanosine diphosphate (GDP) in inactive stage. When the G protein is activated, Gα releases GDP and binds to guanosine triphosphate (GTP) to activate the Gα subunit. Subsequently, Gα separates from Gβ and Gγ, and Gα activates adenylyl cyclase (AC). Subsequently, AC produces cyclic adenosine monophosphate (cAMP), using adenosine triphosphate (ATP). Finally, the cAMP binds with the cAMP-dependent protein kinase A (PKA), which releases and activates the catalytic subunits. These subunits phosphorylate various enzymes to mediate cellular response. Continuous exposure of ractopamine in βAR leads to decreased response. There are three steps occurring from decreasing response to recovering response: (1) phosphorylation, (2) internalization, and (3) and resensitization.

Over prolonged reaction with βAR, β-adrenergic receptor kinase 1 (βARK1), which is the protein phosphorylating the receptor, inactivates the βAR leading to inactivation of βAR-G protein-AC signaling process (phosphorylation). Subsequently, βARK1 binding enables βAR to facilitate with inhibitor protein called β-arrestin, which internalizes into the intracytoplasmic space diminishing the βAR levels in the membrane surface (internalization). The internalized βAR is then dephosphorylated, β-arrestin dissociates, and βAR can be relocated back to the membrane surface (resensitization).

1.3.1.3. Metabolic response of ractopamine

The ractopamine stimulates muscle cells leading to lean muscle mass accretion and decrease in adipose tissue accumulation. Bergen et al. (1989) observed greater fractional protein synthesis rate in semitendinosus muscles of ractopamine-fed pigs than in their counterparts from control pigs. The study concluded that the greater fractional protein synthesis rate in ractopamine-fed pigs could account for the observed muscle hypertrophy. Grant et al. (1993) studied the abundance of α-actin mRNA, which is associated with muscle protein synthesis, in longissimus muscles from ractopamine-fed (20 ppm) and control pigs and observed that the α-actin mRNA was more abundant in ractopamine-fed animals compared to controls. Similar results were reported by the investigations of Smith et al. (1989), in which myosin light chain-1/3 increased in beef longissimus muscle in response to increasing dose of ractopamine.

Several studies reported that ractopamine changes the distribution of βAR subtypes. Winterholler et al. (2007) examined the gene expression in semimembranosus muscles from ractopamine-fed steers and observed that ractopamine did not affect the abundance of β1AR and β3AR mRNA, whereas it increased the expression of β2AR mRNA. Similarly, Gonzalez et al. (2008) found that in beef cattle ractopamine increased β2AR mRNA expression in semimembranosus, but not in longissimus muscle.

Ractopamine supplementation shifts the muscle fiber type from oxidative to glycolytic. Gonzalez et al. (2009) compared myosin heavy chains (MyHC) isoforms in muscles (longissimus lumborum, semimembranosus, adductor, gracilis, vastus lateralis, and rectus femoris) from control and ractopamine-fed beef cattle and

observed that all the muscles demonstrated a significant shift in m fiber type distribution from type I to type IIA, except semimembranosus. In pigs, Depreux et al. (2002) reported that semitendinosus and longissimus muscles exhibited a muscle fiber shift from type IIA and IIX to type IIB in response to ractopamine feeding. Another study demonstrated that the muscle fiber type shifted from intermediate (type IIA) to white muscle (type IIB) in ractopamine-fed pigs compared to control (Aalhus et al., 1992).

Mersmann (1998) noted that βAR increases lipolysis through stimulation of adipocyte triacylglycerol degradation and inhibits synthesis of fatty acids and triacylglycerol in several species. Halsey et al. (2011) examined gene expression of ractopamine-fed pork lean tissue and found that ractopamine reduced the expression of genes associated in lipid synthesis, but enhanced the expression of genes related to oxidative metabolism. Furthermore, ractopamine decreased insulin binding in swine adipocytes in vitro (Liu and Mills, 1990). Mills et al. (2003) observed that ractopamine stimulated lipolysis through both β1AR and β2AR, but β1AR was only partially stimulated compared to β2AR. However, Spurlock et al. (1994) observed that prolonged ractopamine feeding decreases the density of βAR in pork adipose tissue, whereas the density of βAR in skeletal muscle was not affected. The authors claimed that the decrease of βAR may attenuate ractopamine's response in adipose tissue.

1.3.1.4. Influence of ractopamine on growth performance and skeletal muscle development

Ractopamine is classified as beta adrenergic agonist, which functions as a

repartitioning agent redirecting nutrients away from adipose tissue and towards lean muscle (Almeida et al., 2012).

Armstrong et al. (2004) studied the effect of ractopamine (0, 5, 10, and 20 ppm) feeding for different duration (6 to 34 d) in pigs and found that, regardless of the level, ractopamine-fed animals demonstrated improved growth performance (average daily gain and feed efficiency) and carcass characteristics (hot carcass weight, longissimus muscle area, and predicted fat-free carcass lean) than control animals. In addition, feed efficiency was greater for pigs fed 20 ppm ractopamine for 20, 27, and 34 d compared to controls and animals receiving 5 ppm supplementation. Hot carcass weight, longissimus muscle area, and fat-free carcass lean were greater in animals receiving 20 ppm ractopamine supplementation for 34 d than in controls. Overall, higher levels of ractopamine with longer duration improved feed efficiency, hot carcass weight, longissimus muscle area, and predicted fat-free carcass lean.

Rikard-Bell et al. (2009) studied the influence of ractopamine feeding (5 mg/kg from 0 d to 14 d, and 10 mg/kg from 15 d to 31 d) in gilts, boars, and immunologically castrated boars. The results indicated that ractopamine-fed pigs exhibited improved body weight, average daily feed intake, average daily gain, and feed efficiency than controls. Additionally, ractopamine decreased fat tissue deposition in all sexes, especially in immunocastrated and intact males.

Crome et al. (1996) evaluated the effects of feeding ractopamine (0, 10, and 20 ppm) at different finishing weights (68 to 107 kg vs. 85 to 125 kg) and documented that ractopamine-fed animals exhibited greater average daily gain and feed efficiency than controls in each weight group. Moreover, ractopamine-fed groups demonstrated greater longissimus muscle area and lower 10^{th} rib fat than

controls. Overall, ractopamine feeding, regardless of levels and different finishing weights, had positive effects on the growth performance, carcass characteristics, and meat cutting yields.

In beef cattle, Abney et al. (2007) studied the effects of dietary ractopamine (0, 100, and 200 mg/steer/d) and duration of feeding (28, 35, and 42 d). Ractopamine supplementation linearly increased final body weight, average daily gain, and feed efficiency. The results indicated that increasing the dose of ractopamine from 0 to 200 ppm and duration of feeding from 28 to 42 days improved the overall performance. In a separate experiment within the same study, the authors observed that steers fed 200 ppm ractopamine for 30 days exhibited greater longissimus muscle area compared to animals fed 0 ppm ractopamine.

Boler et al. (2012) studied the impact of high dosages of ractopamine hydrochloride (0, 200 and 300 mg/steer/d) on early weaned steers during 28 d feeding and reported that steers receiving 200 and 300 ppm ractopamine had increased live weight and hot carcass weight than controls. Furthermore, feed conversion and average daily gain were greater in ractopamine-fed cattle compared to the controls. While longissimus muscle area was greater in ractopamine-treated animals than controls, there were no differences between the 200 ppm and 300 ppm ractopamine groups.

Recent meta-analyses of ractopamine supplementation studies strengthen the conclusions of the previous investigations that feeding ractopamine is beneficial to growth performance. Pompeu et al. (2017) meta-analyzed 57 previous published studies on ractopamine-fed pigs (between 2004 and 2016) and reported that final weight, average daily gain, and feed efficiency were linearly increased by the level of

ractopamine in diet. Additionally, hot carcass weight and loin depth increased with an increase in ractopamine level. Furthermore, ractopamine supplementation was more effective in pigs with higher initial weight, and barrows were more responsive to ractopamine than gilts. Another meta-analysis study (Apple et al., 2007) of ractopamine feeding in pigs (on 23 articles from early 1990's to 2005) demonstrated that regardless of dietary inclusion level, ractopamine-fed pigs had improved growth performance (average daily feed intake, average daily gain, and feed efficiency) and carcass weight than controls. Meta-analysis of data from studies on ractopamine supplemented beef animals indicated that the beta-agonist improved final body weight, average daily gain, and feed efficiency, hot carcass weight, longissimus muscle area, whereas marbling score, fat thickness at 12^{th} rib, and shear force were negatively impacted by ractopamine (Lean et al., 2014).

1.3.1.5. Influence of ractopamine on meat quality

Although considerable numbers of studies have been published on the impact of ractopamine on meat quality, the results remain inconsistent.

Needham and Hoffman (2015) studied the effects of feeding 10 mg/kg ractopamine to pigs for 28 d on quality of longissimus thoracis and reported an increase in shear force and decrease in *a** value (redness) in ractopamine-fed animals compared to controls. Nonetheless, the differences in shear force and redness were minor and might be negligible when evaluated by a consumer. Rincker et al. (2009) reported that 5 ppm ractopamine supplementation in pigs for 28 days resulted in minimal decrease in color (*L**, *a**, and *b** values) and minimal increase in pH of longissimus muscle, whereas the beta agonist had no impact on shear force

and sensory attributes (juiciness, tenderness, and flavor). Armstrong et al. (2004) reported that 5 or 10 ppm supplementation of ractopamine in pigs for duration of 6 to 34 d did not affect the color of longissimus muscle. However, feeding 10 or 20 ppm ractopamine for 34 d of decreased marbling score of longissimus muscle. Boler et al. (2011) reported that 7.4 mg/kg of ractopamine supplementation in pigs for 27 d increased pH of 6 muscles (semimembranosus, adductor, rectus femoris, vastus lateralis, semitendinosus, and biceps femoris). However, the increase of pH was not sufficiently enough to affect binding strength or protein interactions in further processed hams. Xiong et al. (2006) documented that ractopamine supplementation (20 ppm) in pigs increased the shear force of longissimus muscle, but adequate aging (over 10 days) diminished the shear force differences between ractopamine-fed and control animals.

Ractopamine feeding (200 or 300 mg/steer/d) for 28 d in early weaned beef steers increased shear force in longissimus muscles in comparison with the non-ractopamine fed steers, but aging for more than 14 days diminished the magnitude of shear force differences (Boler et al., 2012). In addition, Bohrer et al. (2014) observed the shear force was greater in ractopamine fed (300 mg/steer/d for 35 d) beef than in non-ractopamine fed group, but meat color, pH, and marbling score were not affected by ractopamine. In contrast, no difference was observed in shear force, marbling score, and color (L^*, a^*, and b^* values) of beef longissimus muscle when 400 mg/steer/d of ractopamine was supplemented for 28 d (Edenburn et al., 2016).

The meta-analysis of data from ractopamine feeding studies in pigs suggested that this growth promotant did not influence postmortem pH and meat color

(Pompeu et al., 2017) and exerted no deleterious effects on pork firmness, water-holding capacity, and marbling score (Apple et al., 2007). However, meta-analysis of data from ractopamine supplementation studies in beef cattle reported that ractopamine increased shear force and decreased marbling score (Lean et al., 2014).

1.3.2. Vitamin E

Vitamin E (α-tocopherol) is lipid-soluble antioxidant that inhibits peroxidation of polyunsaturated fatty acids in plasma membranes (Buttriss and Diplock, 1988). Among the different isomers of vitamin E, α-tocopherol is the chemically and biologically most active form (Carr et al., 2000). The vitamin E was discovered (Evans and Bishop, 1922) as an important biological factor preventing sterility in rats. In the modern day animal agriculture, vitamin E has been used as a supplement for improving fresh meat color stability.

1.3.2.1. Structure and radical scavenging actions of vitamin E

Vitamin E has eight isoforms, namely α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols (Niki and Traber, 2012). Tocopherols are substituted with a phytyl side chain at the position 2 of the chromanol ring, whereas tocotrienols have three double bonds at positions 3, 7, and 11 in the side chain. Tocopherol isomers are classified based on the numbers and positions of methyl group on the chromanol head. Compared to other isoforms, α- form is the most active in radical scavenging (Niki et al., 1986; Yoshida et al., 2003; Niki, 2014). Notably, α-tocopherol is most biologically active than other tocopherols (Yamauchi, 1997; Traber and Atkinson, 2007). Natural tocopherols from plants contain only RRR stereoisomer, whereas

synthetic tocopherols contain all possible stereoisomers at positions 2, 4, and 8 (RRR, SRR, RSR, RRS, SSR, SRS, RSS, and SSS) in equimolar concentrations (Niki, 2014).

Lipid peroxidation has three stages, which are initiation, propagation, and termination. In the initiation stage, carbon-centered lipid radicals (R•) are produced from polyunsaturated fatty acids (RH), which lose hydrogen atoms through a variety of catalyzing elements such as heat, light, and transition metals. Subsequently, in what is known as propagation stage, lipid radicals react with oxygen generating peroxyl radicals (ROO•) that attack other polyunsaturated fatty acids and produce lipid hydroperoxide (ROOH). The chain reactions contibue until the free radicals are combined with other radicals to form non-radical products (RR and ROOR) in the termination stage. Vitamin E interferes with the propagation of lipid peroxidation and scavenges lipid peroxyl radical (ROO•), which attacks lipids (RH) to yield lipid hydroperoxide (ROOH) and lipid radical (R•). The resulting vitamin E radical (E•) then either: (1) is reduced by ascorbic acid to regenerate vitamin E; (2) attacks lipid radical or lipid hydroperoxide; (3) reacts with peroxyl radical; or (4) reacts with another vitamin E radical to generate a non-radical stable product (Niki and Traber, 2012).

1.3.2.2. Vitamin E distribution and associated gene expression in skeletal muscle

The distribution of α-tocopherol in the skeletal muscles of supplemented cattle is muscle-specific. Lynch et al. (2000) studied the distribution of α-tocopherol in muscles from beef cattle supplemented with α-tocopherol acetate (3000 mg/d) and observed that the α-tocopherol level was the highest in oxidative muscles (psoas major and gluteus medius), moderate in intermediate muscle (semimembranosus), and lowest in glycolytic muscles (longissimus thoracis and longissimus lumborum). In

a similar study in pigs supplemented with α-tocopherol, O'Sullivan et al. (1997) observed the level of α-tocopherol was the highest in the muscles of thoracic limb, followed by muscles of neck and thorax, pelvic limb, and the lowest in back muscles. Overall, α-tocopherol level was greater in oxidative muscles compared to glycolytic muscles.

Gonzalez-Calvo et al. (2017) studied gene expression to identify novel genes involved in vitamin E metabolism. The study focused on longissimus thoracis muscle and subcutaneous fat from vitamin E supplemented lambs and found that 29 genes were differentially expressed in longissimus thoracis muscles and that the genes were down-regulated in intracellular signaling cascade. The study also documented that vitamin E supplementation affected gene expression in subcutaneous fat; the genes related to lipid biosynthesis process, cholesterol, and sterol and steroid biosynthesis were up-regulated, whereas genes associated with stress response were down-regulated. Furthermore, expression of lipid metabolism related genes in chicken were affected by vitamin E supplementation; cytosolic phospholipase A2 gene (cPLA2) was down-regulated, whereas peroxisome proliterator-activated receptor beta (PPAP-β) and heart fatty acid binding protein genes (H-FABP) were up-regulated (Li et al., 2009). The results of the aforementioned studies indicated that vitamin E supplementation influenced gene expression in fat tissue and skeletal muscles, and some of the affected genes are related to meat quality.

1.3.2.3. Impact of vitamin E on meat quality

It is well known that supplementation of vitamin E minimizes lipid oxidation and improves meat color stability. Faustman et al. (1989) studied the effects of

vitamin E supplementation (370 IU/d) on beef cattle and found that gluteus medius muscle from vitamin E supplemented animals exhibited lower metmyoglobin content and lipid oxidation than their counterparts from controls, and lipid oxidation and metmyoglobin content were highly correlated. Chan et al. (1996) evaluated color stability of three muscles (longissimus lumborum, gluteus medius, and psoas major) from beef cattle supplemented with vitamin E (1204 IU/head/d for 122 days) and reported that color shelf-life of these muscles was prolonged by vitamin E supplementation. These authors also found that supplementation of vitamin E increased α-tocopherol concentration in microsomes and that muscles from vitamin E supplemented animals had low lipid oxidation, oxymyoglobin oxidation, and metmyoglobin formation in microsomes.

Mitsumoto et al. (1993) compared the effect of dietary and postmortem supplementation of vitamin E in beef. The study documented that dietary supplementation of vitamin E (1,500 IU/d) minimized metmyoglobin content and lipid oxidation in ground beef during 9 d display compared to controls, whereas equivalent postmortem exogenous supplementation of vitamin E in ground beef was less effective in preventing lipid oxidation and metmyoglobin content. The study indicated that endogenous vitamin E from diet (incorporated into cellular membranes) worked better than exogenous vitamin E mixed into ground beef and highlighted that stabilization of membrane lipids is critical to improving postmortem stability of muscle lipids and meat color.

Hoving-Bolink et al. (1998) examined longissimus and psoas major muscles from pigs supplemented with vitamin E and found that the muscles from vitamin E treated pigs exhibited lower lipid oxidation than those from control animals.

Interestingly, these authors observed an improved color stability only in longissimus muscle, but not in psoas major. Guo et al. (2006a) studied vitamin E supplementation levels (40, 200, and 400 IU/kg feed) and duration (3, 6, and 9 weeks) in pigs and found that vitamin supplementation did not affect color attributes of longissimus muscle; however, lipid oxidation and drip loss were decreased by vitamin E. Further studies (Guo et al., 2006b) evaluated the effects of vitamin E (40 or 200 IU/kg) on growth performance and quality of pork patties. While no effect was observed in growth performance due to vitamin E supplementation, the antioxidant was beneficial to improving the oxidative stability in fresh and cooked ground ham patties during 6 days storage.

Mercier et al. (1998) studied the effect of different types of fats (rapeseed, tallow, and soya) and vitamin E supplementation (400 ppm) on turkey for 16 weeks and found that regardless of fat source, pectoralis and sartorius muscles from vitamin E-supplemented birds demonstrated lower lipid oxidation than controls. However, vitamin E had no effect on color stability of the muscles during refrigerated storage. Olivo et al. (2001) reported that supplementation of vitamin E may inhibit the onset of pale, soft, exudative (PSE) condition in chicken and may improve meat functional properties because as a membrane antioxidant vitamin E can maximize the antioxidant capacity and increase the physical stability of subcellular membranes.

1.4. Two dimensional gel electrophoresis (2-DE)

1.4.1. Basic concept of 2-DE

Two-dimensional gel electrophoresis (2-DE) is an electrophoretic method that enables analyses of 500-2000 individual proteins extracted from complex tissue

(Bendixen, 2005). The first 2-DE study conducted (MacGillivray and Rickwood, 1974) was the combination of two techniques: electrophoresis (SDS PAGE) and denaturation isoelectric focusing. After the first study, O'Farrell (1975) suggested better techniques. However, 2-DE techniques were initially hampered by low accuracy, poor reproducibility of isoelectric focusing, and the absence of a technique to identify protein spots on 2-DE gels (Rabilloud et al., 2010). In the 1980's, immobilized pH gradients were developed that improved reproducibility in the first dimension, Edman sequencing enabled protein identification, and the development of mass spectrometry increased the speed and sensitivity of protein characterization (Rabilloud and Lelong, 2011).

The basic concept of 2-DE is to separate the individual proteins based on molecular weight and charge. The first step is protein extraction from biological samples, and the chemicals for protein extraction must not alter the proteins' charge. The extracted proteins are then loaded on pH gradient strip for the first dimension isoelectric focusing (IEF). In this stage, proteins reach their pI and will have no net charge. Subsequently, the strip is equilibrated with buffers so that proteins became strongly negative charged. The equilibrated IEF strip is loaded onto SDS-PAGE gel, and the proteins are separated based on their molecular weight (second dimension). After the second dimension, the protein spots are detected by staining solutions, are quantified by image analyses, and are identified with mass spectrometry.

1.4.2. Proteomic studies in meat science using 2-DE

The applications of proteomics in meat science has advanced significantly in the past two decades (D'Alessandro & Zolla, 2013; Gobert et al., 2014; Suman et al.,

2014; Joseph et al., 2015). Several proteomic investigations were undertaken to identify biomarkers for meat quality and characterize the biochemical mechanisms governing the conversion of muscle to meat.

Bouley et al. (2004) investigated proteome mapping of beef semitendinosus to understand the mechanisms controlling postmortem muscle metabolism and meat quality. The authors identified a total of 75 proteins, and these proteins were related to metabolism (25.5%), cell structure (17%), cell defense (16%) and contractile apparatus (14.5%). Subsequently, Chaze et al. (2006) attempted to map alkaline proteins in beef semitendinosus muscle and identified 32 proteins; the identified proteins were related to metabolism (40%), contractile apparatus (15%), and cell structure (15%). Furthermore, several investigations characterized early postmortem (24 h) changes in the proteome of beef longissimus thoracis (Jia et al., 2007) and longissimus dorsi and semitendinosis (Jia et al., 2006) muscles. Bjarnadóttir et al. (2010) identified proteome changes during 48 h postmortem in beef longissimus thoracis muscle, and observed that 26 proteins underwent changes. In the pork longissimus dorsi, the abundance of 18 proteins changed in during 48 h postmortem (Lametsch et al., 2002). Morzel et al. (2004) found changes in 37 proteins (associated in structural and metabolism) during 72 h postmortem in pork longissimus lumborum.

Extensive investigations were undertaken to characterize the role of proteome components in quality attributes of postmortem skeletal muscles. These studies were focused on tenderness (Lametsch et al., 2003; Jia et al., 2009; Guillemin et al., 2011), water holding capacity (Melody et al., 2004; Di Luca et al., 2011; Di Luca et al., 2013), and color (Joseph et al., 2012; Canto et al., 2015; Nair et al., 2016).

These aforementioned studies highlighted the criticality of muscle proteome on quality attributes governing consumers' purchase intent and eating satisfaction.

Recent studies identified proteome changes in postmortem skeletal muscles due to dietary supplementation of growth promotants and other nutrients. Ma et al. (2015) studied the impact of L-arginine supplementation on the proteome of pork longissimus muscle and observed that 18 proteins were differentially expressed between control and L-arginine treated groups, and the differentially proteins were involved in energy metabolism and muscle structure. Additionally, Costa-Lima et al. (2015) studied the effect of ractopamine supplementation on the sarcoplasmic proteome profile of pork longissimus thoracis and found that 9 protein spots were differentially abundant between control and ractopamine-fed groups, and these results suggested that ractopamine influenced proteins involved in glycolytic metabolism in this muscle. Furthermore, Wu et al. (2017) studied the effect of dietary ractopamine on the sarcoplasmic proteome profile of pork semimembranosus muscle and observed that 6 protein spots were differentially abundant between control and ractopamine supplemented group, and the identified proteins were related in oxygen transport, chaperone activity, and plasma membrane repair.

CHAPTER 2

Ractopamine-induced changes in whole-muscle proteome of postmortem beef longissimus lumborum

Abstract

Ractopamine is a beta-adrenergic agonist approved for use in beef cattle and pigs as a repartitioning agent to increase lean muscle deposition and to decrease lipogenesis. Although the effects of dietary ractopamine on proteome profile of postmortem pork muscles have been examined, its influence on beef muscle proteome has not been studied. Therefore, the objective of this study was to examine the effect of ractopamine on the proteome profile of postmortem beef longissimus lumborum (LL) muscle. LL muscle samples were obtained from the carcasses of nine (n = 6) ractopamine-fed (RAC; 400 mg ractopamine hydrochloride for 28 days) and control (CON; diet without ractopamine) beef steers. The whole-muscle proteome was analyzed using two-dimensional gel electrophoresis and tandem mass spectrometry. Five differentially abundant spots were identified, and all the spots were over-abundant in RAC. The identified proteins were involved in muscle structure development (F-actin-capping protein subunit beta-2; PDZ and LIM domain protein-3), chaperone activity (heat shock protein beta-1), oxygen transport (myoglobin), and glycolysis (L-lactate dehydrogenase A chain). These results suggested that dietary ractopamine influences the abundance of enzymes associated with muscle development and muscle fiber type shift in beef LL muscle.

Keyword: beef, longissimus lumborum, ractopamine, whole muscle proteome

2.1. Introduction

Ractopamine is a beta-adrenergic agonist growth promotant approved in the pork and beef industries in the United States. Ractopamine enhances muscle protein synthesis, increases leanness, and decreases lipid deposition (Johnson and Chung, 2007). The improved leanness is due to the increased feed efficiency (Avendaño-Reyes et al., 2006; Abney et al., 2007; Quinn et al., 2016) and muscle accretion (Quinn et al., 2008; Bryant et al., 2010; Boler et al., 2012; Edenburn et al., 2016). Furthermore, several studies documented that ractopamine feeding resulted in muscle fiber type shift from oxidative (type IIA, IIX) to glycolytic (type IIB) in pigs (Depreux et al., 2002; Gunawan et al., 2007; Almeida et al., 2015). Furthermore, Gonzalez et al. (2009) examined six muscles (longissimus lumborum, semimembranosus, vastus lateralis, adductor, gracilis, and rectus femoris) from ractopamine-fed steers and observed a fiber type shift from type I to type IIA in all muscles except semimembranosus. Meta-analyses of data from ractopamine feeding studies in beef animals observed that the growth promotant increased rib eye area and hot carcass weight, but decreased tenderness and marbling (Lean et al., 2014).

Recently, several studies examined the influence of dietary ractopamine on the proteome profile of postmortem skeletal muscles. Costa-Lima et al. (2015) analyzed the sarcoplasmic proteome profile of pork longissimus thoracis and found that nine proteins were differentially abundantly between control and ractopamine-fed pigs; these results suggested that ractopamine influenced the abundance of enzymes associated in glycolytic metabolism and thus may potentially influence the conversion of muscle to meat. Additionally, Wu et al. (2016) examined sarcoplasmic proteome of semimembranosus from pigs fed ractopamine and control diets and

observed that five proteins (involved in oxidative metabolism, chaperone, and plasma membrane repair) were differentially abundant between the two groups.

Although the effects of ractopamine on proteome profile of pork muscles have been studied, its influence on beef muscle proteome has not been investigated. Therefore, the objective of our study was to examine the influence of ractopamine on the proteome of beef longissimus lumborum (LL) muscle.

2.2. Materials and Methods

2.2.1. Animal production and muscle sample collection

The muscle samples were obtained from a feeding study (Edenburn et al., 2016) completed at the University of Illinois. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC protocol #12009) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Steers were fed a corn-based diet for 188 d before the initiation of this study and were implanted with Component TE-IS (80 mg trenbolone acetate, 16 mg estradiol; Elanco Animal Health, Greenfield, IN) 104 d before the initiation of the study. A set of 72 steers were used in the study and were allocated to 12 pens with 6 animals per pen. Pens were randomly assigned to 2 treatments on d 0: control (CON) and ractopamine (RAC). Steers were fed in 3-m concrete bunks. Steers in RAC group received Optaflexx 45 (Elanco Animal Health, Greenfield, IN, USA) to provide 400 mg RAC/steer per day for 28 days. All animals were fed the same basal diet of 60% dry-rolled corn, 20% corn silage (approximately 50:50 grain: forage), 10% dry distillers

grains, and 10% supplement on a dry matter basis; each diet was formulated to meet or exceed NRC guidelines (NRC, 2000). Steers were fed once daily.

At the end of the 28-d ractopamine supplementation, one steer was randomly selected from each of the 6 pens in CON (628 kg average body weight) and RAC (635 kg average body weight) at the end of the 28-day feeding period. This approach provided 6 replicates (n = 6) for proteome analysis. These 12 steers were transported to a USDA-inspected commercial meat packing facility. Cattle were fasted for approximately 16 h, but were provided water until slaughter. At 24 h postmortem, a 2.5-cm thick sample of LL muscle was collected from each carcass, vacuum-packaged, frozen immediately, and transported to University of Kentucky.

2.2.2. Isolation of whole-muscle proteome

The whole-muscle proteome from beef LL muscle was extracted as described by Lametsch et al. (2003). One gram of frozen muscle tissue was cut and homogenized in 5 mL extraction buffer (8M urea, 2M Thiourea, 2% Chaps, 65 mM DTT, and 0.5% pH 3-10 ampholyte). Crude extract was transferred to centrifuge tubes, vigorously shaken for 2 h at 4°C, and centrifuged (10,000 × g) for 30 min at 4°C. The supernatant consisting of muscle proteins was filtered and utilized for analysis.

2.2.3. Two-dimensional electrophoresis (2-DE)

Bradford assay was used to determine the protein concentration of sarcoplasmic extract (Bio-Rad, Hercules, CA, USA). The sarcoplasmic proteins (900 µg) were mixed with rehydration buffer (Bio-Rad) optimized to 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% Bio-Lyte 5/8 ampholyte, and 0.001% of Bromophenol blue.

The mixture was loaded into immobilized pH gradient (IPG, pH 3-10, 17 cm, Bio-Rad). Gels were subjected to passive rehydration for 16 h. First dimension isoelectric focusing (IEF) process was conducted using Protean IEF cell system. First, an active rehydration step was conducted with low voltage (50 V) and increased voltage by stages, with final rapid voltage ramping to reach a total of 80 kVh. Subsequently, the IPG strips were equilibrated with equilibration buffer I (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2% DTT) for 15 min, followed by equilibration buffer II (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2.5% Iodoacetamide) for 15 min. The second dimension protein separation process was conducted by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 38.5:1 ratio of acrylamide to bis-acrylamide) using Protean II XL system (Bio-Rad). The gels were stained by Colloidal Coomassie Blue for 48 h, and destained until the background of the gels was cleared. The CONT and RAC samples were evaluated under the same conditions (two gels/sample) resulting in 24 gels.

2.2.4. Gel image analysis

The gels were scanned using VersaDoc (Bio-Rad) and gel images were analyzed using PDQuest (Bio-Rad, Hercules, CA, USA). Firstly, spot detection was conducted and matched, then normalized (Joseph et al., 2012). The spots were considered differentially abundant when a 1.5-fold or more intensity difference was measured between CONT and VITE, with 90% statistical significance ($P < 0.10$) in a pairwise Student's t-test.

2.2.5. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS)

Protein spots differentially abundant between CONT and VITE were subjected to mass spectrometric identification. The protein spots were removed from the gels and then subjected to dithiothreitol reduction, iodoacetamide alkylation, and in-gel trypsin digestion. The peptides formed were extracted and concentrated. Subsequently the peptides were injected for nano-LC-MS/MS analysis using LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with an Eksigent Nanoflex cHiPLC™ system (Eksigent, Dublin, CA, USA) through a nano electrospray ionization source. A reverse phase cHiPLC column (75 µm × 150 mm) was operated (300 nL/min flow rate) for separation of the peptides. Water with 0.1% (v/v) formic acid was used for mobile phase A, and acetonitrile with 0.1% (v/v) formic acid was used for mobile phase B. A 50 min gradient was applied; initial 3% mobile phase B was linearly increased to 50% in 24 min and further to 85% and 95% for 5 min each before it was decreased to 3%, then the column was re-equilibrated. The mass analysis method was consisted of eight scan events per segment. The first scan event was an Orbitrap MS scan (100-1600 m/z) with 60,000 resolutions for parent ions, and then followed by data dependent MS/MS for fragmentation of the 7 most intense ions through collision induced dissociation (CID).

2.2.6. MS/MS protein identification

The LC-MS/MS data were submitted to a local Mascot server for MS/MS protein identification through Proteome Discoverer (version 1.3, Thermo Fisher Scientific, Waltham, MA, USA) based on the *Bos taurus* database from National

Center for Biotechnology Information (NCBI). The parameters of the MASCOT MS/MS ion search were: trypsin digest with a maximum of two miscleavages, cysteine carbamidomethylation, methionine oxidation, a maximum of 10 ppm MS error tolerance, and a maximum of 0.8 Da MS/MS error tolerance. A decoy database was conducted and searched. To distribute the confidence indicators for the peptide matches, filter settings to determine false discovery rates (FDR) were used.. Peptide matches that passed the filter associated with the strict FDR (target setting of 0.01) were assigned as high confidence. For the MS/MS ion search, proteins with two of more high confidence peptides were considered unambiguous identifications without manual inspection, whereas proteins identified with one high confidence peptide were manually inspected and confirmed.

2.3. Results and Discussion

Five differentially abundant spots were identified from the image analyses of whole muscle proteome gels (Figure 1). The accession number, database score, matched peptides, and sequence coverage of the identified proteins are listed in table 1. All the identified proteins were over-abundant ($P < 0.05$) in RAC (Table 2). The proteins present in these spots were involved in muscle contraction (F-actin-capping protein subunit beta 2; PDZ and LIM domain protein 3), chaperone (heat shock protein beta-1), transport (myoglobin), and glycolysis (L-lactate dehydrogenase A chain).

2.3.1. F-actin-capping protein subunit β2 (CapZ β2)

F-actin-capping protein subunit β2 belongs to the actin-capping protein family

(Pyle et al., 2002). The functions of actin-capping proteins are binding the barbed ends of actin filaments, controlling length of actin filaments, and stabilizing actin filaments (Clark et al., 2002; Pollard and Cooper, 1986; Russel et al., 2010). Actin-capping protein β has two isoforms (β1 and β2), of which the β2 isoform is found at the cell-cell junctions and is concentrated at the intercalated disc, whereas β1 isoform is found in Z-disk of myofibrils (Schafer et al., 1994). Since dietary ractopamine increases skeletal muscle growth and protein accretion, the over-abundance of CapZ β2 protein in RAC is logical (Wang et al., 2014).

Several proteomic studies examined the possible relationships between actin-capping protein and quality attributes in pork and beef. Ponsuksili et al. (2009) suggested that CapZB gene is a candidate gene for pork quality. Lametsch et al. (2003) compared the proteomes of pork longissimus dorsi aged for 72 hours and non-aged counterparts to characterize the relationship between proteome components and tenderness, and observed that the abundance of capping protein was greater in the samples aged for 72 hours than in the non-aged pork. Guillemin et al. (2011) examined biomarkers for tenderness in longissimus and semitendinosus muscles from Charolais steers and young bulls and observed that CapZ proteins were over-abundant in semitendinosus muscles of steers compared to their counterparts from bulls. Gagaoua et al. (2015) attempted to identify the biomarkers for beef tenderness using longissimus thoracis and semitendinosus muscles of young bulls of three continental breeds (Aberdeen Angus, Blond d'Aquitaine and Limousin) and documented that Aberdeen Angus had greater levels of CapZβ than the other two breeds; however, no relationship between tenderness and CapZβ was observed.

2.3.2. PDZ and LIM domain protein-3 (ALP)

PDZ and LIM domain protein is a protein motif, which plays a critical role in organ development and mediates signals between nucleus and cytoskeleton (Krcmery et al., 2010). PDZ and LIM domain protein-3 is known as α-actinin-associated LIM domain protein (ALP), which consists of PDZ domain in the amino terminus and a single LIM domain at the carboxy terminus (Hoshijima, 2006; Krcmery et al., 2010). Several studies indicated that ALP is involved in muscle development. Pomies et al. (1999) observed that ALP is expressed in muscle cells and is up-regulated during muscle differentiation, and concluded that ALP interacts with α-actinin to stabilize and strengthen the contractile structure of muscle cells. Furthermore, ALP subfamily genes are functionally involved in muscle differentiation (Pomies et al., 2007; Wang et al., 2010), and the ALP strengthens the association between α-actinin and actin filaments (Xia et al., 1997; Klaavuniemi et al., 2004; Vallenius et al., 2004).

2.3.3. Heat shock protein beta-1 (Hsp27)

Heat shock protein beta-1 (Hsp27) belongs to the family of small heat shock proteins, which are distributed widely in various tissues and plays an important role in cell survival under stress conditions (Bakthisaran et al., 2015; Haslbeck and Vierling, 2015). This protein plays a critical role in stabilizing cytoskeleton, especially with protecting muscle filaments and stabilizing the muscle structure (Perng et al., 1999a; Perng et al., 1999b). Furthermore, Pivovarova et al. (2005) reported that Hsp27 efficiently prevent heat-induced aggregation of F-actin.

Previous proteomic investigations indicated that Hsp27 is involved in

muscle growth and meat quality. Lametsch et al. (2006) analyzed the proteome of longissimus muscles from pigs exhibiting compensatory growth and normal growth and observed that Hsp27 was over-abundant in animals demonstrating compensatory growth. These findings suggested an important role of Hsp27 in muscle hypertrophy during compensatory growth. Shibata et al. (2009) compared proteome of semitendinosus muscles from grass-fed and grain-fed cattle and found that Hsp27 was over-abundant in grain-fed cattle. These authors speculated that Hsp27 may have some role in skeletal muscle growth in exercise-restricted cattle. Furthermore, Hsp27 has been reported to be associated with tenderness (Kim et al., 2008; Carvalho et al., 2014) and color (Sayd et al., 2006; Joseph et al., 2012).

2.3.4. Myoglobin

Myoglobin is the oxygen-binding heme protein in mammalian in muscle tissue, and its primary functions are storage and transport of oxygen (Lehninger et al., 2005; Schiaffino and Reggiani, 2011). Myoglobin transports oxygen from red blood cells to mitochondria within the muscles during periods of increased metabolic activity and serves as an oxygen reservoir during anoxic and hypoxic conditions in the skeletal muscles (Ordway and Garry, 2004). Furthermore, myoglobin plays a critical role of meat color (Mancini and Hunt, 2005; Suman and Joseph, 2013; Faustman and Suman, 2017). The over-abundance of myoglobin in RAC was unexpected since dietary ractopamine is known to cause a muscle fiber shift form oxidative to glycolytic in pigs (Depreux et al., 2002). The increased abundance of myoglobin may possibly be a mechanism to compensate the oxygen demand to generate energy (i.e., ATP) for the β-adrenergic agonist receptor signaling pathways in increased muscle

development (Barros et al., 1999).

2.3.5. L-Lactate dehydrogenase A chain

L-Lactate dehydrogenase A chain is an enzyme catalyzing the reversible conversion of lactate to pyruvate (Gladden, 2004). The over-abundance of lactate dehydrogenase could be attributed to the muscle fiber shift from oxidative to glycolytic caused by ractopamine. Previous studies reported that lactate dehydrogenase is more abundant in glycolytic muscle fibers than in oxidative muscle fibers, and lactate dehydrogenase is more active in glycolytic than in oxidative muscles (Picard et al., 2002; Huber et al., 2007; Izumiya et al., 2008). Ractopamine shifts muscle fiber type from fast oxidative-glycolytic (type IIA and IIX) to fast glycolytic (type IIB) in pigs (Depreux et al., 2002) and from slow oxidative (type I) to fast oxidative-glycolytic (type IIA) in cattle (Gonzalez et al., 2008). Furthermore, Costa-Lima et al. (2015) have observed over-abundance of L-lactate dehydrogenase A chain in longissimus thoracis muscles of ractopamine-fed pigs.

Meta-analysis of studies on ractopamine-fed beef reported that this beta agonist increases shear force (Lean et al., 2014). The over-abundance of L-lactate dehydrogenase A chain may be one of the possible biomarker for the decrease in meat tenderness in ractopamine-fed cattle. Guillemin et al. (2011) studied 24 protein markers related to meat tenderness in comparison between beef longissimus thoracis (fast oxidative-glycolytic) and semitendinosus (fast glycolytic) muscles, and found that lactate dehydrogenase B chain was positively correlated with toughness in semitendinosus, but not in longissimus thoracis. Furthermore, Maltin et al. (2003) argued in their review that glycolytic muscles would be less tender than oxidative

ones due to inherent variations in muscle fiber size, contractile mechanisms, and metabolic pathways.

2.4. Conclusions

The results of the present study suggested that dietary ractopamine influenced the abundance of proteins related to muscle structure development, chaperone activity, oxygen transport, and glycolysis in postmortem beef longissimus lumborum muscle. Additional studies are necessary to characterize how ractopamine influences the proteome in ante- and peri-mortem beef skeletal muscles to characterize the influence of growth promotant on muscle to meat conversion and meat quality attributes.

Figure 2.1. Coomassie-stained two-dimensional gel of whole muscle proteome extracted from longissimus lumborum of beef steers fed on ractopamine. Five protein spots differentially abundant in control and ractopamine-fed beef steers are encircled and numbered

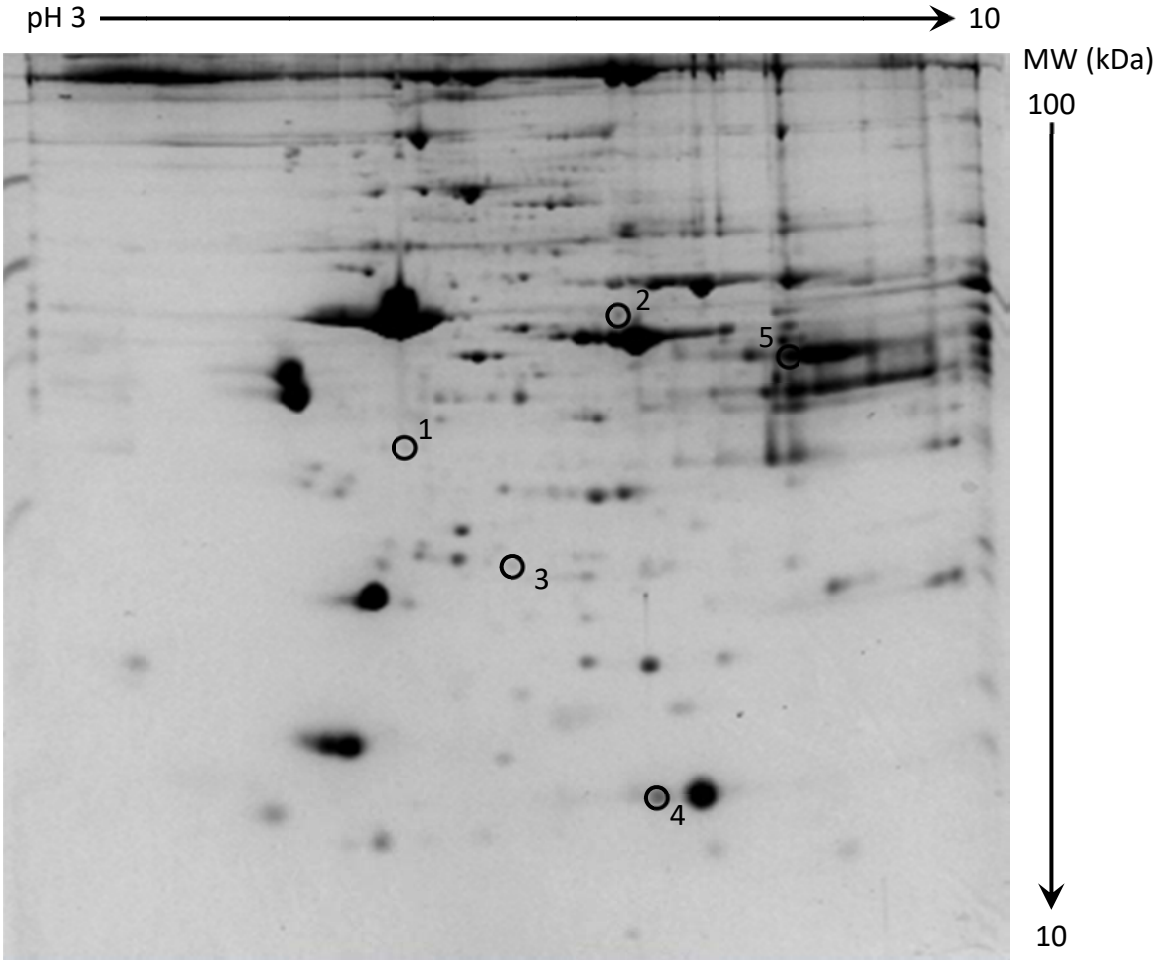

Table 2.1. Differentially abundant proteins in whole muscle proteome of longissimus lumborum from beef steers fed on ractopamine

Spot [a]	Protein	Accession number	ProtScore/ matched peptides	Sequence coverage (%)
1	F-actin-capping protein subunit beta-2	P79136-2	1569.72/19	62.13
2	PDZ and LIM domain protein-3	Q3SYZ8	1346.65/20	63.92
3	Heat shock protein beta-1	Q3T149	2787.86/20	80.60
4	Myoglobin	P02192	2371.06/26	99.35
5	L-lactate dehydrogenase A chain	P19858	944.61/22	56.33

Each spots were identified by accession number (UniProt), ProtScore, matched peptides number, and sequence coverage of peptides

[a] Spot numbers were based on gel image (Figure 2.1)

Table 2.2. Functional roles of differentially abundant proteins in whole muscle proteome of longissimus lumborum from beef steers fed on ractopamine

Spot [a]	Protein	Function	Over-abundant treatment [b]	Spot ratio [c]
1	F-actin-capping protein subunit beta-2	Muscle development	RAC	2.20
2	PDZ and LIM domain protein-3	Muscle development	RAC	1.80
3	Heat shock protein beta-1	Chaperone activity	RAC	1.96
4	Myoglobin	Oxygen transport	RAC	1.51
5	L-lactate dehydrogenase A chain	Glycolysis	RAC	1.62

[a] Spot numbers were based on gel image (Figure 2.1)

[b] CON = Non-ractopamine hydrochloride diet ; RAC = 400 mg ractopamine hydrochloride diet for 28 days before slaughter

[c] Spot ratio of RAC/CON

CHAPTER 3

Supranutritional supplementation of vitamin E influences sarcoplasmic proteome profile of postmortem beef longissimus lumborum muscle

Abstract

Vitamin E is a lipid-soluble antioxidant that inhibits lipid oxidation and improves beef color stability. The molecular aspects of the effect of vitamin E on fresh beef color, from the standpoint of lipid oxidation-induced myoglobin oxidation, have been extensively studied. In contrast, the influence of vitamin E on sarcoplasmic proteome profile of beef skeletal muscles is yet to be investigated. Therefore, the objective of this study was to examine the effect of dietary vitamin E on sarcoplasmic proteome of postmortem beef longissimus lumborum (LL) muscle. Beef LL muscle samples (24 h postmortem) were obtained from the carcasses of nine (n = 9) vitamin E-fed (VITE; 2,000 IU vitamin E for 89 days) and control (CONT; diet without supplemental vitamin E) heifers. The sarcoplasmic proteome was analyzed using two-dimensional gel electrophoresis and image analyses. Five differentially abundant spots were identified using mass spectrometry, and all the spots were over-abundant in CONT. The proteins in the differentially abundant spots were antioxidant proteins (thioredoxin-dependent peroxide reductase, peroxiredoxin-6, and serum albumin) and glycolytic enzymes (beta-enolase and triosephosphate isomerase). The antioxidant proteins minimize oxidation of lipids and proteins in muscle matrix, whereas the glycolytic enzymes generate NADPH, which helps maintain the antioxidant proteins in their reduced forms. These findings suggested that the strong antioxidant activity of vitamin E leads to less expression of antioxidant proteins and antioxidant related-proteins in beef skeletal muscles.

Keywords: antioxidant proteins, glycolytic enzymes, sarcoplasmic proteome

3.1. Introduction

Color is the most important attribute influencing customers' beef purchase decisions (Mancini and Hunt, 2005; Suman et al., 2014). Various exogenous and endogenous factors contribute to myoglobin redox stability and color stability in fresh red meats (Suman and Nair, 2017; Neethling et al., 2017). Lipid oxidation is a major endogenous factor contributing to myoglobin oxidation and fresh meat discoloration (Faustman et al., 2010; Suman and Joseph, 2013). Vitamin E (α-tocopherol) is a lipid-soluble antioxidant that inhibits peroxidation of polyunsaturated fatty acids in plasma membranes (Buttriss and Diplock, 1988). Extensive research documented that supranutritional supplementation of vitamin E to beef cattle improved steak color and lipid stability (Faustman et al., 1989; Arnold et al., 1992; Chan et al., 1996; Smith et al., 1996). From this standpoint, vitamin E supplementation has been employed globally as an efficient pre-harvest strategy to improve color and lipid stabilities in fresh beef (Faustman et al., 1998).

Postmortem addition of exogenous vitamin E to ground beef in amounts equivalent to those deposited by dietary supplementation was ineffective in minimizing myoglobin and lipid oxidation (Mitsumoto et al., 1993). These observations indicated that endogenous vitamin E incorporated into cellular membranes (via dietary delivery) functioned better than exogenous vitamin E mixed into ground beef. Lynch et al. (2000) studied the distribution of α-tocopherol in muscles from vitamin E-fed cattle and observed that the highest concentration was in oxidative muscles (e.g. psoas major), whereas the lowest concentration was in glycolytic muscles (e.g. longissimus thoracis).

The molecular basis for the effects of vitamin E on beef color and myoglobin

redox stability has been extensively studied using mass spectrometry, and these investigations focused on the mechanistic interactions between reactive lipid oxidation products and myoglobin in postmortem skeletal muscles (Lynch et al., 1998; Alderton et al., 2003; Suman et al., 2007; Yin et al., 2011). Numerous metabolic and antioxidant proteins in the sarcoplasm are critically involved in the biochemical mechanisms governing fresh beef color stability (Joseph et al., 2012; Canto et al., 2015; Nair et al., 2016; Nair et al., 2018). Nonetheless, investigations were not undertaken on the potential influence of vitamin E on the sarcoplasmic proteome components, which continuously interact with myoglobin. Therefore, the objective of this study was to identify the influence of dietary vitamin E on sarcoplasmic proteome of postmortem beef longissimus lumborum (LL) muscle.

3.2. Materials and Methods

3.2.1. Animal production and muscle sample collection

The muscle samples were obtained from a feeding study (Harsh et al., 2017) completed at the University of Illinois. All protocols were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #15008).

Eighteen Angus × Simmental heifers were used in a randomized complete block design with treatment factors including daily dietary inclusion of no supplemental (CONT) or 2,000 IU Vitamin E/animal per day (VITE). Heifers were managed as a group on a trace mineral maintenance diet prior to trial initiation and were administered an implant of 140 mg trenbolone acetate and 14 mg estradiol (Component TE-H; Elanco Animal Health, Greenfield, IN). After being weighed on day 0 and 1, heifers were stratified by body weight (n = 9 heifers per treatment). Diets

were the same for both dietary treatments with the exception of vitamin E inclusion as feed supplement. Diets were formulated to meet or exceed NRC (2000) recommendations and contained 20% corn silage, 35% modified wet distillers grains with solubles, 35% dry rolled corn, and 10% supplement (dry matter basis). Dietary vitamin E (dl-alpha-tocopheryl acetate) was provided to VITE animals, and individual feed intakes of all animals were collected using a GrowSafe feeding system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Heifers were weighed at 28 d intervals and fed for ad libitum intake daily for a total of 89 d on feed. Heifers were housed in 4.88 m × 4.88 m pens in a confinement barn with slatted, concrete floors covered by interlocking rubber matting.

On d 90, heifers were humanely slaughtered under USDA inspection at a commercial slaughter facility. At 24 h postmortem, a 2.54-cm section of longissimus lumborum (LL) was excised from between the 12^{th} and 13^{th} rib section of the carcasses, immediately vacuum-packaged, frozen, and shipped to the University of Kentucky.

3.2.2. Isolation of sarcoplasmic proteome

The sarcoplasmic proteome from beef LL muscle was extracted as described by Joseph et al. (2012). Five grams of frozen muscle tissue was thawed and homogenized in 25 mL ice-cold extraction buffer (40 mM Tris, 2 mM EDTA, and pH 8.0). The homogenized sample was centrifuged at 10,000 × g for 10 min at 4 °C, and the supernatant (sarcoplasmic proteome) was filtered and utilized.

3.2.3. Two-dimensional electrophoresis (2-DE)

Bradford assay was used to determine the protein concentration of sarcoplasmic extract (Bio-Rad, Hercules, CA, USA). The sarcoplasmic proteins (900 μg) were mixed with rehydration buffer (Bio-Rad) optimized to 7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, 0.5% Bio-Lyte 5/8 ampholyte, and 0.001% of Bromophenol blue. The mixture was loaded into immobilized pH gradient (IPG, pH 3-10, 17 cm, Bio-Rad). Gels were subjected to passive rehydration for 16 h. First dimension isoelectric focusing (IEF) process was conducted using Protean IEF cell system. First, an active rehydration step was conducted with low voltage (50 V) and increased voltage by stages, with final rapid voltage ramping to reach a total of 80 kVh. Subsequently, the IPG strips were equilibrated with equilibration buffer I (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2% DTT) for 15 min, followed by equilibration buffer II (6 M urea, 0.375 M Tris–HCl, pH 8.8, 2% SDS, 20% glycerol, 2.5% Iodoacetamide) for 15 min. The second dimension protein separation process was conducted by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 38.5:1 ratio of acrylamide to bis-acrylamide) using Protean II XL system (Bio-Rad). The gels were stained by Colloidal Coomassie Blue for 48 h, and destained until the background of the gels was cleared. The CONT and VITE samples were evaluated under the same conditions (two gels/sample) resulting in 36 gels.

3.2.4. Gel image analysis

The gels were scanned using VersaDoc (Bio-Rad) and gel images were analyzed using PDQuest (Bio-Rad, Hercules, CA, USA). Firstly, spot detection was conducted and matched, then normalized (Joseph et al., 2012). The spots were

considered differentially abundant when a 1.5-fold or more intensity difference was measured between CONT and VITE, with 90% statistical significance ($P < 0.10$) in a pairwise Student's t-test.

3.2.5. Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS)

Protein spots differentially abundant between CONT and VITE were subjected to mass spectrometric identification. The protein spots were removed from the gels and then subjected to dithiothreitol reduction, iodoacetamide alkylation, and in-gel trypsin digestion. The peptides formed were extracted and concentrated. Subsequently the peptides were injected for nano-LC-MS/MS analysis using LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with an Eksigent Nanoflex cHiPLC™ system (Eksigent, Dublin, CA, USA) through a nano electrospray ionization source. A reverse phase cHiPLC column (75 µm × 150 mm) was used (300 nL/min flow rate) for separation of the peptides. Water with 0.1% (v/v) formic acid was used for mobile phase A, and acetonitrile with 0.1% (v/v) formic acid was used for mobile phase B. A 50 min gradient was applied; initial 3% mobile phase B was linearly increased to 50% in 24 min and further to 85% and 95% for 5 min each before it was decreased to 3%, then the column was re-equilibrated. The mass analysis method consisted of eight scan events per segment. The first scan event was an Orbitrap MS scan (100-1600 m/z) with 60,000 resolutions for parent ions, and then followed by data dependent MS/MS for fragmentation of the 7 most intense ions through collision induced dissociation (CID).

3.2.6. MS/MS protein identification

The LC-MS/MS data were submitted to a local Mascot server for MS/MS protein identification through Proteome Discoverer (version 1.3, Thermo Fisher Scientific, Waltham, MA, USA) based on the *Bos taurus* database from National Center for Biotechnology Information (NCBI). The parameters of the MASCOT MS/MS ion search were: trypsin digest with a maximum of two miscleavages, cysteine carbamidomethylation, methionine oxidation, a maximum of 10 ppm MS error tolerance, and a maximum of 0.8 Da MS/MS error tolerance. A decoy database was conducted and searched. To distribute the confidence indicators for the peptide matches, filter settings to determine false discovery rates (FDR) were used. Peptide matches that passed the filter associated with the strict FDR (target setting of 0.01) were assigned as high confidence. For the MS/MS ion search, proteins with two of more high confidence peptides were considered unambiguous identifications without manual inspection, whereas proteins identified with one high confidence peptide were manually inspected and confirmed.

3.3. Results and Discussion

Five differentially abundant spots were identified from the gel image analyses (Figure 1). The identified proteins are listed in table 1 along with their accession number, score of the database search (ProtScore), matched peptides, and sequence coverage. All the identified proteins were over-abundant ($P < 0.05$) in CONT than in VITE (Table 2). The differentially abundant proteins were involved in antioxidant mechanisms (thioredoxin-dependent peroxide reductase, peroxiredoxin-6, and serum albumin) and glycolysis (beta-enolase and triosephosphate isomerase).

Interestingly, antioxidant proteins were over-abundant in CONT, and it appears that vitamin E supplementation provided antioxidant protection to muscle tissue resulting in low expression of endogenous antioxidant proteins in VITE than in CONT.

3.3.1. Antioxidant proteins

Three antioxidant proteins were over-abundant in CONT. These proteins were peroxiredoxins (thioredoxin-dependent peroxide reductase and peroxiredoxin-6) and serum albumin.

Peroxiredoxins are cysteine-dependent peroxidase enzymes, which play critical roles in reducing peroxides in cells (Karplus, 2015; Perkins et al., 2015). Mammalian peroxiredoxins (PRDX) have six isomers (i.e., PRDX 1 through 6). Thioredoxin-dependent peroxide reductase (PRDX3) is a 2-Cys peroxiredoxin and is abundant in mitochondria, whereas peroxiredoxin-6 (PRDX6) is a 1-Cys peroxiredoxin and is abundant in cytosol (Rhee et al., 2012). The main function of PRDX is antioxidant activity (Jacobson et al., 1989; Chae et al., 1999; Cox et al., 2009). PDRX reduces the natural hydroperoxides (ROOH) through peroxidase activity (Wood et al., 2003). Furthermore, Poynton and Hampton (2014) suggested that PDRX can be used as biomarker for oxidative stress.

The low abundance of PRDX3 and PRDX6 in VIT may be due to the strong antioxidant effects of α-tocopherol. Vitamin E scavenges peroxyl radicals, which attack lipid to yield lipid hydroperoxides and lipid radicals, and eventually block lipid peroxidation (Brigelius-Flohe, 2009). Furthermore, α-tocopherol is the important lipophilic radical-scavenging antioxidant in-vivo (Niki, 2014). Therefore, over-abundance of PRDX in CONT group could be attributed to the absence of strong

antioxidant protection against the peroxyl radicals.

Due to their antioxidant activity, previous studies reported that several members of PRDX protein family are related to meat color and tenderness. Wu et al. (2016) compared color stability of longissimus lumborum and psoas major muscles (15 d postmortem) from Chinese Luxi yellow cattle and observed the presence of PRDX2 proteins in both muscles; however, *a** value (surface redness) was positively correlated to the level of PRDX2 in LL, whereas it was negatively correlated to the abundance of PRDX2 in PM. Furthermore, PRDX3 and PRDX6 were identified in PM muscle, and both proteins were negatively correlated to metmyoglobin reducing activity. Similarly, Gagaoua et al. (2015) examined 21 proteins in longissimus thoracis muscles from young bulls and documented that PRDX6 was positively correlated to *a** value, which may be explained by the antioxidant and phospholipase activities of this protein. Furthermore, peroxiredoxin proteins were found to be related to meat tenderness during early postmortem. Jia et al. (2009) studied the 7-day postmortem beef longissimus thoracis muscles separated into tender and tough groups based on shear force values and compared proteomes in both groups using muscle samples biopsied 4 days before slaughter and samples collected 1 h postmortem. The protein PRDX6 was found only in the samples from tender group (antemortem and postmortem), and the authors concluded that PRDX6 might be a potential biomarker for beef tenderness. Similarly, Grabez et al. (2015) categorized early postmortem beef semimembranosus muscles into two group (tender vs. tough) based on shear force and observed that PRDX3 was over-abundant in the mitochondrial proteome of tender group. Furthermore, Polati et al. (2012) studied proteomic changes in beef longissimus dorsi muscle during prolonged aging, and PRDX1 was observed to

decrease from day 0 to 12 and increased from day 12 to 26 postmortem. The authors explained that the changes in the level of PRDX1 is possibly because the postmortem muscle is under stress, during which the redox potential of the cells is very variable, and peroxiredoxins are possibly involved in these cellular changes.

Serum albumin is a protein for the transport and storage of numerous endogenous and exogenous biomolecules, and it is the most abundant protein in blood plasma (Kragh-Hansen, 1981). Bovine serum albumin is a single polypeptide chain, 66 kDa of molecular weight, and consists of 583 amino acid residues that shares approximately 76% sequence homology with human serum albumin (Anand and Mukherjee, 2013). The antioxidant activity of serum albumin is the result of its ligand-binding property. Serum albumin is well known for its ability to bind a variety of bioactive molecules, including metal ions such as iron and copper known to promote oxidation (Roche et al., 2008). The over-abundance of serum albumin in CONT also may be due to the absence of vitamin E, which is a strong antioxidant (Niki, 2014).

Several proteomic studies reported the presence of serum albumin in fresh as well as cooked meats. Montowska and Pospiech (2013) studied muscle proteins that are stable during aging and thermal processing of different red meats and poultry, and observed that bovine serum albumin is minimally degraded in cooked beef and is a relatively thermostable protein, which may be used as a biomarker for authentication of meat products. Costa-Lima et al. (2015) compared sarcoplasmic proteome of longissimus thoracis muscles from ractopamine-fed and control pigs and reported that serum albumin was over-abundant in the ractopamine-fed pork. These authors argued that the increased abundance of serum albumin is possibly to protect

muscle proteins against reactive products of lipolysis caused by dietary ractopamine. Di Luca et al. (2013) evaluated proteomics of water holding capacity in pork longissimus thoracis et lumborum muscle, and reported that the abundance of serum albumin was lower in high drip loss samples compared to the samples from low drip loss and intermediate drip loss groups.

3.3.2. Glycolytic proteins

Two glycolytic proteins (beta-enolase and triosephosphate isomerase) were over-abundant in CONT. Beta-enolase is a muscle-specific enolase, which is abundant in skeletal muscles, and is one of three enolase isoforms (Tracy and Hedges, 2000). Beta-enolase catalyzes the interconversion of 2-phosphoglycerate and phosphoenolpyruvate (Peshavaria and Day, 1991). Triosephosphate isomerase is another glycolytic enzyme, which catalyzes the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate (Blacklow et al., 1988). Increased levels of these glycolytic enzymes increase the activity of pentose phosphate pathway generating NADPH, which in turn helps maintain the antioxidant enzymes such as peroxiredoxins and glutathione peroxidase in their reduced forms (Kondoh et al., 2007). Therefore, the over-abundance of glycolytic enzymes in CONT may be a cellular mechanism to maintain the antioxidant proteins in their reduced state to counterbalance the oxidative stress.

Several proteomic investigations reported the relationship between glycolytic enzymes and meat quality attributes. Di Luca et al. (2013) compared three groups of pork longissimus thoracis et lumborum muscles exhibiting varying degrees of drip loss (i.e., high drip, intermediate, and low drip losses) and observed that

triosephosphate isomerase was over-abundant in low drip loss samples indicating that this protein may be utilized as a biological marker for water holding capacity. Hwang et al. (2005) studied postmortem proteome changes in pork longissimus muscle (days 1, 3, and 7) and found that triosephosphate isomerase was positively correlated to shear force. Furthermore, Jia et al. (2006) analyzed proteome changes in early postmortem (0 h and 24 h) beef longissimus dorsi and semitendinosis muscles and observed that triosephosphate isomerase decreased in longissimus dorsi muscle during 24 h postmortem. Lametsch et al. (2003) compared proteome changes in pork longissimus dorsi muscle between 0 and 72 h postmortem to identify proteins correlated to shear force and observed that triosephosphate isomerase was over-abundant in 72 h postmortem samples and the protein was negatively correlated to shear force.

3.4. Conclusions

The results of the present study suggested that dietary vitamin E influenced the abundance of sarcoplasmic proteins related to antioxidant activity and glycolysis in postmortem beef LL muscle. The strong antioxidant protection offered by vitamin E could have led to low expression of antioxidant proteins as well as glycolytic enzymes that generate antioxidant metabolites in the VITE group, whereas the lack of such protection in CONT group may have led to increased expression of these proteins in the skeletal muscles. Further studies should examine the influence of vitamin E on the muscle proteome in live animals as well as perimortem muscles to understand how this antioxidant modulates oxidative stability of skeletal muscles.

Figure 3.1. Coomassie-stained two-dimensional gel of sarcoplasmic proteome extracted from longissimus lumborum muscle of beef heifers fed on vitamin E. Five protein spots differentially abundant in control and vitamin E-fed heifers are encircled and numbered

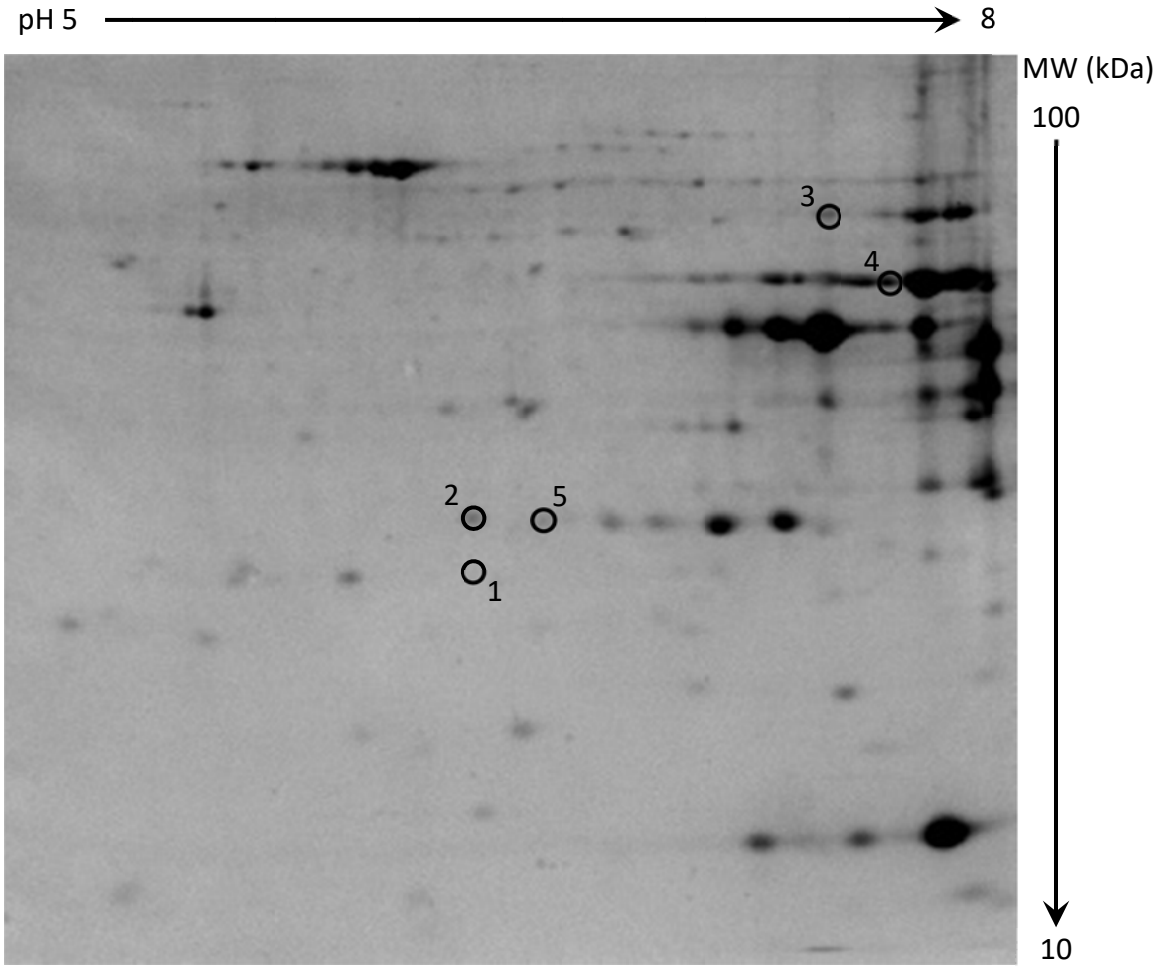

Table 3.1. Differentially abundant proteins in sarcoplasmic proteome of longissimus lumborum muscle from beef heifers fed on vitamin E

Spot [a]	Protein	Accession number	ProtScore/ matched peptides	Sequence coverage (%)
1	Thioredoxin-dependent peroxide reductase, mitochondrial	P35705	370.20/12	49.81
2	Peroxiredoxin-6	O77834	1428.21/23	73.66
3	Serum albumin	P02769	2285.61/54	76.94
4	Beta-enolase	Q3ZC09	10787.41/63	79.26
5	Triosephosphate isomerase	Q5E956	2113.08/30	96.39

Each spots were identified by accession number (UniProt), ProtScore, matched peptides number, and sequence coverage of peptides

[a] Spot numbers were based on gel image (Figure 3.1)

Table 3.2. Functional roles of differentially abundant proteins in sarcoplasmic proteome of longissimus lumborum from beef heifers fed on vitamin E

Spot [a]	Protein	Function	Over-abundant treatment [b]	Spot ratio [c]
1	Thioredoxin-dependent peroxide reductase, mitochondrial	Antioxidant activity	CONT	2.15
2	Peroxiredoxin-6	Antioxidant activity	CONT	1.51
3	Serum albumin	Antioxidant activity	CONT	1.59
4	Beta-enolase	Glycolysis	CONT	2.23
5	Triosephosphate isomerase	Glycolysis	CONT	1.71

[a] Spot numbers were based on gel image (Figure 3.1)

[b] CONT = Non-vitamin E diet ; VITE = 2,000 IU vitamin E diet for 89 days

[c] Spot ratio of CONT/VITE

SUMMARY

Global meat production is continuously increasing. Numerous pre-harvest strategies are employed in animal agriculture to achieve the increasing demand in meat production. Supplementation of feed ingredients is one approach to enhance animal growth and meat production. Ractopamine is a beta-adrenergic agonist repartitioning agent, which redirects nutrients away from lipid tissue and towards lean muscle deposition in cattle and pigs. Vitamin E is a natural antioxidant that protects muscle lipids from peroxidation and improves beef color stability. While the effects of ractopamine and vitamin E supplementation on physicochemical and biochemical aspects of fresh beef quality have been investigated extensively, studies are yet to be undertaken on the impact of these dietary ingredients on the proteome profiles of postmortem beef skeletal muscles. Therefore, the objectives of this research were to identify the effects of dietary ractopamine and vitamin E on proteome profile of postmortem beef longissimus lumborum (LL) muscle.

In the first experiment, the effects of ractopamine on whole muscle proteome profile of beef LL were examined. The LL muscle samples were obtained from the carcasses of six (n = 6) ractopamine-fed (RAC; 400 mg ractopamine hydrochloride for 28 days) and control (CON; diet without ractopamine) beef steers. The muscle proteins were separated by two-dimensional electrophoresis (2-DE) and were identified using mass spectrometry. The results indicated that 5 proteins were differentially abundant between RAC and CON groups, and all the identified proteins were over-abundant in RAC. The differentially abundant proteins were related to muscle structure development (F-actin-capping protein subunit beta-2, PDZ and LIM

domain protein-3), chaperone (heat shock protein beta-1), oxygen transportation (myoglobin), and glycolysis (L-lactate dehydrogenase A chain). The results suggested that supplementation of ractopamine influences abundance of proteins associated with muscle development and fiber type shift in beef LL muscle.

The second experiment examined the effects of supranutritional supplementation of vitamin E on sarcoplasmic proteome profile of beef LL muscle. Beef LL samples (24 h postmortem) were obtained from the carcasses of nine (n = 9) vitamin E-fed (VITE; 2,000 IU vitamin E for 89 days) and control (CONT; diet without supplemental vitamin E) heifers. The sarcoplasmic proteins were separated by 2-DE and were identified using mass spectrometry. The results indicated that 5 proteins were differentially abundant between VITE and CONT groups. All the differentially proteins were over-abundant in the CONT group. The identified proteins were associated with antioxidant activity (thioredoxin-dependent peroxide reductase, peroxiredoxin-6, and serum albumin) and glycolysis (beta-enolase and triosephosphate isomerase). While the antioxidant proteins protect biomolecules from oxidation, the glycolytic enzymes generate NADPH that maintains the antioxidant proteins in reduced state. These results suggested that the strong antioxidant activity of vitamin E leads to low expression of antioxidant and antioxidant-related proteins in beef LL muscle.

In summary, supplementation of ractopamine and vitamin E affected proteome profiles of beef LL muscle. Ractopamine increased the abundance of proteins related to muscle development and fiber type shift, whereas supranutritional supplementation of vitamin E resulted in low abundance of antioxidant proteins. These findings indicated that dietary ingredients influenced

proteome profile of postmortem beef LL muscle, and the changes in the abundance of muscle proteins are closely related to the biological functions of the supplements.

REFERENCES

Aalhus, J.L., Schaefer, A.L., Murray, A.C. & Jones, S.D.M., 1992. The effect of ractopamine on myofibre distribution and morphology and their relation to meat quality in swine. Meat Sci. 31, 397-409.

Abney, C.S., Vasconcelos, J.T., McMeniman, J.P., Keyser, S.A., Wilson, K.R., Vogel, G.J. & Galyean, M.L., 2007. Effects of ractopamine hydrochloride on performance, rate and variation in feed intake, and acid-base balance in feedlot cattle. J. Anim. Sci. 85, 3090-3098.

Aïssat, D., Sosa, J.M., Avila, D.M.D., Bertrand, K.P. & Reeves, J.J., 2002. Endocrine, growth, and carcass characteristics of bulls immunized against luteinizing hormone-releasing hormone fusion proteins. J. Anim. Sci. 80, 2209-2213.

Alderton, A.L., Faustman, C., Liebler, D.C. & Hill, D.W., 2003. Induction of redox instability of bovine myoglobin by adduction with 4-hydroxy-2-nonenal. Biochemistry. 42, 4398-4405.

Almeida, V.V., Nuñez, A.J.C. & Miyada, V.S., 2012. Ractopamine as a metabolic modifier feed additive for finishing pigs: a review. Braz. Arch. Biol. Techn. 55, 445-456.

Almeida, V.V., Nuñez, A.J.C., Schinckel, A.P., Ward, M.G., Andrade, C., Sbardella, M., Berenchtein, B., Coutinho, L.L. & Miyada, V.S., 2015. Gene expression of beta-adrenergic receptors and myosin heavy chain isoforms induced by ractopamine feeding duration in pigs not carrying the ryanodine receptor mutation. Livest. Sci. 172, 91-95.

Amatayakul-Chantler, S., Jackson, J.A., Stegner, J., King, V., Rubio, L.M.S., Howard, R.,

Lopez, E. & Walker, J., 2012. Immunocastration of Bos indicus × Brown Swiss bulls in feedlot with gonadotropin-releasing hormone vaccine Bopriva provides improved performance and meat quality. J. Anim. Sci. 90, 3718-3728.

Anand, U. & Mukherjee, S., 2013. Binding, unfolding and refolding dynamics of serum albumins. BBA-Gen. Subjects 1830, 5394-5404.

Anderson, D.B., Moody, D.E. & Hancock, D.L., 2004. Beta adrenergic agonists. In: Encyclopedia of Animal Science. Eds: Pond, W.G. & Bell, A.W., Marcel-Dekker Inc., New York, USA. pp. 104-107.

Apple, J., Rincker, P.J., McKeith, F.K., Carr, S.N., Armstrong, T.A. & Matzat, P.D., 2007. Review: meta-analysis of the ractopamine response in finishing swine. Prof. Anim. Sci. 23, 179-196.

Armstrong, T.A., Ivers, D.J., Wagner, J.R., Anderson, D.B., Weldon, W.C. & Berg, E.P., 2004. The effect of dietary ractopamine concentration and duration of feeding on growth performance, carcass characteristics, and meat quality of finishing pigs. J. Anim. Sci. 82, 3245-3253.

Arnold, R.N., Scheller, K.K, Arp, S.C, Williams, S.N., Buege, D.R. & Schaefer, D.M., 1992. Effect of long- or short-term feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability. J. Anim. Sci. 70, 3055-3065.

Arp, T.S., Howard, S.T., Woerner, D.R., Scanga, J.A., McKenna, D.R., Kolath, W.H., Chapman, P.L., Tatum, J.D. & Belk, K.E., 2014. Effects of dietary ractopamine hydrochloride and zilpaterol hydrochloride supplementation on performance, carcass traits, and carcass cutability in beef steers. J. Anim. Sci. 92, 836-843.

Avendaño-Reyes, L., Torres-Rodríguez, V., Meraz-Murillo, F.J., Pérez-Linares, C.,

Figueroa-Saavedra, F. & Robinson, P.H., 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84, 3259-3265.

Bakthisaran, R., Tangirala, R. & Rao, C.M., 2015. Small heat shock proteins: role in cellular functions and pathology. BBA-Proteins Proteom. 1854, 291-319.

Barros, R.D.A., Okoshi, M.P. & Cicogna, A.C., 1999. Beta-adrenergic pathway in healthy and hypertrophied hearts. Arq. Bras. Cardiol. 72, 649-656.

Batorek, N., Čandek-Potokar, M., Bonneau, M. & Van Milgen, J., 2012. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6, 1330-1338.

Beermann, D.H., 2002. Beta-adrenergic receptor agonist modulation of skeletal muscle growth. J. Anim. Sci. 80, E18-E23.

Bendixen, E., 2005. The use of proteomics in meat science. Meat Sci. 71, 138-149.

Bergen, W.G., Johnson, S.E., Skjaerlund, D.M., Babiker, A.S., Ames, N.K., Merkel, R.A. & Anderson, D.B., 1989. Muscle protein metabolism in finishing pigs fed ractopamine. J. Anim. Sci. 67, 2255-2262.

Bjarnadóttir, S.G., Hollung, K., Færgestad, E.M. & Veiseth-Kent, E., 2010. Proteome changes in bovine longissimus thoracis muscle during the first 48 h postmortem: shifts in energy status and myofibrillar stability. J. Agr. Food Chem. 58, 7408-7414.

Blacklow, S.C., Raines, R.T., Lim, W.A., Zamore, P.D. & Knowles, J.R., 1988. Triosephosphate isomerase catalysis is diffusion controlled. Biochemistry-US 27, 1158-1165.

Bohrer, B.M., Edenburn, B.M., Boler, D.D., Dilger, A.C. & Felix, T.L., 2014. Effect of

feeding ractopamine hydrochloride (Optaflexx) with or without supplemental zinc and chromium propionate on growth performance, carcass characteristics, and meat quality of finishing steers. J. Anim. Sci. 92, 3988-3996.

Bohrer, B.M., Kyle, J.M., Boler, D.D., Rincker, P.J., Ritter, M.J. & Carr, S.N., 2013. Meta-analysis of the effects of ractopamine hydrochloride on carcass cutability and primal yields of finishing pigs. J. Anim. Sci. 91, 1015-1023.

Boler, D.D., Holmer, S.F., Duncan, D.A., Carr, S.N., Ritter, M.J., Stites, C.R., Petry, D.B., Hinson, R.B., Allee, G.L., McKeith, F.K. & Killefer, J., 2011. Fresh meat and further processing characteristics of ham muscles from finishing pigs fed ractopamine hydrochloride. J. Anim. Sci. 89, 210-220.

Boler, D.D., Shreck, A.L., Faulkner, D.B., Killefer, J., McKeith, F.K., Homm, J.W. & Scanga, J.A., 2012. Effect of ractopamine hydrochloride (Optaflexx) dose on live animal performance, carcass characteristics and tenderness in early weaned beef steers. Meat Sci. 92, 458-463.

Bouley, J., Chambon, C. & Picard, B., 2004. Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. Proteomics 4, 1811-1824.

Brigelius-Flohé, R., 2009. Vitamin E: The shrew waiting to be tamed. Free Radical Bio. Med. 46, 543-554.

Bryant, T.C., Engle, T.E., Galyean, M.L., Wagner, J.J., Tatum, J.D., Anthony, R.V. & Laudert, S.B., 2010. Effects of ractopamine and trenbolone acetate implants with or without estradiol on growth performance, carcass characteristics, adipogenic enzyme activity, and blood metabolites in feedlot steers and

heifers. J. Anim. Sci. 88, 4102-4119.

Buttriss, J.L. & Diplock, A.T., 1988. The relationship between α-tocopherol and phospholipid fatty acids in rat liver subcellular membrane fractions. BBA-Lipid Lipid Met. 962, 81-90.

Canto, A.C.V.C.S., Suman, S.P., Nair, M.N., Li, S., Rentfrow, G., Beach, C.M., Silva, T.J.P., Wheeler, T.L., Shackelford, S.D., Grayson, A., McKeith, R.O. & King, D.A., 2015. Differential abundance of sarcoplasmic proteome explains animal effect on beef longissimus lumborum color stability. Meat Sci. 102, 90-98.

Carr, A.C., Zhu, B.Z. & Frei, B., 2000. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E). Circ. Res. 87, 349-354.

Carron, J., Morel, C., Hammon, H.M. & Blum, J.W., 2005. Ontogenetic development of mRNA levels and binding sites of hepatic β-adrenergic receptors in cattle. Domest. Anim. Endocrin. 28, 320-330.

Carvalho, M.E., Gasparin, G., Poleti, M.D., Rosa, A.F., Balieiro, J.C.C., Labate, C.A., Nassu, R.T., Tullio, R.R., Regitano, L.C.D.A., Mourão, G.B. & Coutinho, L.L., 2014. Heat shock and structural proteins associated with meat tenderness in Nellore beef cattle, a Bos indicus breed. Meat Sci. 96, 1318-1324.

Chae, H.Z., Kim, H.J., Kang, S.W. & Rhee, S.G., 1999. Characterization of three isoforms of mammalian peroxiredoxin that reduce peroxides in the presence of thioredoxin. Diabetes Res. Clin. Pr. 45, 101-112.

Chan, W.K.M., Hakkarainen, K., Faustman, C., Schaefer, D.M., Scheller, K.K. & Liu, Q., 1996. Dietary vitamin E effect on color stability and sensory assessment of spoilage in three beef muscles. Meat Sci. 42, 387-399.

Chaze, T., Bouley, J., Chambon, C., Barboiron, C. & Picard, B., 2006. Mapping of

alkaline proteins in bovine skeletal muscle. Proteomics 6, 2571-2575.

Clark, K.A., McElhinny, A.S., Beckerle, M.C. & Gregorio, C.C., 2002. Striated muscle cytoarchitecture: an intricate web of form and function. Annu. Rev. Cell Dev. Bi. 18, 637-706.

Cook, R.B., Popp, J.D., Kastelic, J.P., Robbins, S. & Harland, R., 2000. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J. Anim. Sci. 78, 2778-2783.

Costa-Lima, B.R.C., Suman, S.P., Li S., Beach, C.M., Silva, T.J.P., Silveira, E.T.F., Bohrer, B.M. & Boler, D.D., 2015. Dietary ractopamine influences sarcoplasmic proteome profile of pork longissimus thoracis. Meat Sci. 103, 7-12.

Cox, A.G., Peskin, A.V., Paton, L.N., Winterbourn, C.C. & Hampton, M.B., 2009. Redox potential and peroxide reactivity of human peroxiredoxin 3. Biochemistry-US 48, 6495-6501.

Crome, P.K., McKeith, F.K., Carr, T.R., Jones, D.J., Mowrey, D.H. & Cannon, J.E., 1996. Effect of ractopamine on growth performance, carcass composition, and cutting yields of pigs slaughtered at 107 and 125 kilograms. J. Anim. Sci. 74, 709-716.

D'Alessandro, A. & Zolla, L., 2013. Meat science: from proteomics to integrated omics towards system biology. J. Proteomics 78, 558-577.

Depreux, F.F.S., Grant, A.L., Anderson, D.B. & Gerrard, D.E., 2002. Paylean alters myosin heavy chain isoform content in pig muscle. J. Anim. Sci. 80, 1888-1894.

Di Luca, A., Elia, G., Hamill, R. & Mullen, A.M., 2013. 2D DIGE proteomic analysis of early post mortem muscle exudate highlights the importance of the stress

response for improved water-holding capacity of fresh pork meat. Proteomics 13, 1528-1544.

Di Luca, A., Mullen, A.M., Elia, G. Davey, G. & Hamill, R.M., 2011. Centrifugal drip is an accessible source for protein indicators of pork ageing and water-holding capacity. Meat Sci. 88, 261-270.

Dikeman, M.E., 2007. Effects of metabolic modifiers on carcass traits and meat quality. Meat Sci. 77, 121-135.

Duckett, S.K. & Pratt, S.L., 2014. Meat science and muscle biology symposium - anabolic implants and meat quality. J. Anim. Sci. 92, 3-9.

Dunshea, F.R., Allison, J.R.D., Bertram, M., Boler, D.D., Brossard, L., Campbell, R., Crane, J.P., Hennessy, D.P., Huber, L., de Lange, C., Ferguson, N., Matzat, P., McKeith, F., Moraes, P.J.U., Mullan, B.P., Noblet, J., Quiniou, N. & Tokach, M., 2013. The effect of immunization against GnRF on nutrient requirements of male pigs: a review. Animal 7, 1769-1778.

Dunshea, F.R., Colantoni, C., Howard, K., McCauley, I., Jackson, P., Long, K.A., Lopaticki, S., Nugent, E.A., Simons, J.A., Walker, J. & Hennessy, D.P., 2001. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. J. Anim. Sci. 79, 2524-2535.

Dunshea, F.R., D'Souza, D.N. & Channon, H.A., 2016. Metabolic modifiers as performance-enhancing technologies for livestock production. Anim. Front. 6, 6-14.

Dunshea, F.R., D'Souza, D.N., Pethick, D.W., Harper, G.S. & Warner, R.D., 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71, 8-38.

Dunshea, F.R., King, R.H., Campbell, R.G., Sainz, R.D. & Kim, Y.S., 1993. Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. J. Anim. Sci. 71, 2919-2930.

Edenburn, B.M, Kneeskern, S.G., Bohrer, B.M., Rounds, W., Boler, D.D., Dilger, A.C. & Felix, T.L., 2016. Effects of supplementing zinc or chromium to finishing steers fed ractopamine hydrochloride on growth performance, carcass characteristics, and meat quality. J. Anim. Sci. 94, 771-779.

Evans, H.M. & Bishop, K.S., 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 56, 650-651.

FASS, 2010. Guide for the care and use of agricultural animals in agricultural research and teaching (3rd ed.). Federation of Animal Science Societies, Champaign, Illinois, USA.

Faustman, C. & Suman, S.P., 2017. The eating quality of meat: color In: Lawrie´s Meat Science (8th ed.). Ed: Toldrá, F., Woodhead Publ. Ltd. Cambridge, England. pp. 329-356.

Faustman, C., Cassens, R.G., Schaefer, D.M., Buege, D.R., Williams, S.N. & Scheller, K.K., 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54, 858-862.

Faustman, C., Chan, W.K., Schaefer, D.M. & Havens, A., 1998. Beef color update: the role for vitamin E. J. Anim. Sci. 76, 1019-1026.

Faustman, C., Sun, Q., Mancini, R. & Suman, S.P., 2010. Myoglobin and lipid oxidation interactions: mechanistic bases and control. Meat Sci. 86, 86-94.

FDA, 2000. New animal drugs for use in animal feeds; ractopamine hydrochloride. Fed. Regist. 65, 4111-4112.

FDA, 2006. New animal drugs; zilpaterol. Fed. Regist. 71, 53005-53006.

Gagaoua, M., Terlouw, E.M.C., Boudjellal, A., & Picard, B., 2015. Coherent correlation networks among protein biomarkers of beef tenderness: What they reveal. J. Proteomics 128, 365-374.

Gagaoua, M., Terlouw, E.M.C., Micol, D., Boudjellal, A., Hocquette, J.F. & Picard, B., 2015. Understanding early post-mortem biochemical processes underlying meat color and pH decline in the longissimus thoracis muscle of young blond d'aquitaine bulls using protein biomarkers. J. Agr. Food Chem. 63, 6799-6809.

Gladden, L.B., 2004. Lactate metabolism: a new paradigm for the third millennium. J. Physiol. 558, 5-30.

Gobert, M., Sayd, T., Gatellier, P. & Santé-Lhoutellier, V., 2014. Application to proteomics to understand and modify meat quality. Meat Sci. 98, 539-543.

Gonzalez-Calvo, L., Dervishi, E., Joy, M., Sarto, P., Martin-Hernandez, R., Serrano, M., Ordovas, J.M. & Calvo, J.H., 2017. Genome-wide expression profiling in muscle and subcutaneous fat of lambs in response to the intake of concentrate supplemented with vitamin E. BMC Genomics 18, 92.

Gonzalez, J.M., Dijkhuis, R.D., Johnson, D.D., Carter, J.N. & Johnson, S.E., 2008. Differential response of cull cow muscles to the hypertrophic actions of ractopamine-hydrogen chloride. J. Anim. Sci. 86, 3568-3574.

Gonzalez, J.M., Johnson, S.E., Thrift, T.A., Savell, J.D., Ouellette, S.E. & Johnson, D.D., 2009. Effect of ractopamine-hydrochloride on the fiber type distribution and shelf-life of six muscles of steers. J. Anim. Sci. 87, 1764-1771.

Grabež, V., Kathri, M., Phung, V., Moe, K.M., Slinde, E., Skaugen, M., Saarem, K. & Egelandsdal, B., 2015. Protein expression and oxygen consumption rate of

early postmortem mitochondria relate to meat tenderness. J. Anim. Sci. 93, 1967-1979.

Grant, A.L., Skjaerlund, D.M., Helferich, W.G., Bergen, W.G. & Merkel, R.A., 1993. Skeletal muscle growth and expression of skeletal muscle alpha-actin mRNA and insulin-like growth factor I mRNA in pigs during feeding and withdrawal of ractopamine. J. Anim. Sci. 71, 3319-3326.

Guillemin, N., Bonnet, M., Jurie, C. & Picard, B. 2011. Functional analysis of beef tenderness. J. Proteomics 75, 352-365.

Gunawan, A.M., Richert, B.T., Schinckel, A.P., Grant, A.L. & Gerrard, D.E., 2007. Ractopamine induces differential gene expression in porcine skeletal muscles. J. Anim. Sci. 85, 2115-2124.

Guo, Q., Richert, B.T., Burgess, J.R., Webel, D.M., Orr, D.E., Blair, M., Fitzner, G.E., Hall, D.D., Grant, A.L. & Gerrard, D.E., 2006a. Effects of dietary vitamin E and fat supplementation on pork quality. J. Anim. Sci. 84, 3089-3099.

Guo, Q., Richert, B.T., Burgess, J.R., Webel, D.M., Orr, D.E., Blair, M., Grant, A.L. & Gerrard, D.E., 2006b. Effect of dietary vitamin E supplementation and feeding period on pork quality. J. Anim. Sci. 84, 3071-3078.

Halsey, C.H.C., Weber, P.S., Reiter, S.S., Stronach, B.N., Bartosh, J.L. & Bergen, W.G., 2011. The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. J. Anim. Sci. 89, 1011-1019.

Hancock, D.L, Wagner, J.F. & Anderson, D.B., 1991. Effects of estrogens and androgens on animal growth. In: Growth regulation in farm animals. Eds: Pearson, A.M. & Dutson T.R., Elsevier Appl. Sci., New York, USA. pp. 255-297.

Harsh B.N., Dilger, A.C., Boler, D.D. & Shike D.W., 2017. Effects of a multi-element

trace mineral injection and vitamin E supplementation on performance, carcass characteristics, and color stability of strip steaks from feedlot heifers. J. Anim. Sci. (In press)

Haslbeck, M. & Vierling, E., 2015. A first line of stress defense: small heat shock proteins and their function in protein homeostasis. J. Mol. Biol. 427, 1537-1548.

Hoshijima, M., 2006. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. Am. J. Physiol.-Heart C. 290, H1313-H1325.

Hoving-Bolink, A.H., Eikelenboom, G., van Diepen, J.Th.M., Jongbloed, A.W. & Houben, J.H., 1998. Effect of dietary vitamin E supplementation on pork quality. Meat Sci. 49, 205-212.

Huber, K., Petzold, J., Rehfeldt, C., Ender, K. & Fiedler, I., 2007. Muscle energy metabolism: structural and functional features in different types of porcine striated muscles. J. Muscle Res. Cell M. 28, 249-258.

Huxsoll, C.C., Price, E.O. & Adams, T.E., 1998. Testis function, carcass traits, and aggressive behavior of beef bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 76, 1760-1766.

Hwang, I.H., Park, B.Y., Kim, J.H., Cho, S.H. & Lee, J.M., 2005. Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. Meat Sci. 69, 79-91.

Izumiya, Y., Hopkins, T., Morris, C., Sato, K., Zeng, L., Viereck, J., Hamilton, J.A., Ouchi, N., LeBrasseur, N.K. & Walsh, K., 2008. Fast/glycolytic muscle fiber growth reduces fat mass and improves metabolic parameters in obese mice. Cell

Metab. 7, 159-172.

Jacobson, F.S., Morgan, R., Christman, M. & Ames, B., 1989. An alkyl hydroperoxide reductase from Salmonella typhimurium involved in the defense of DNA against oxidative damage. Purification and properties. J. Biol. Chem. 264, 1488-1496.

Janett, F., Gerig, T., Tschuor, A.C., Amatayakul-Chantler, S., Walker, J., Howard, R., Bollwein, H. & Thun, R., 2012. Vaccination against gonadotropin-releasing factor (GnRF) with Bopriva significantly decreases testicular development, serum testosterone levels and physical activity in pubertal bulls. Theriogenology 78, 182-188.

Jaros, P., Bürgi, E., Stärk, K.D.C., Claus, R., Hennessy, D. & Thun, R., 2005. Effect of active immunization against GnRH on androstenone concentration, growth performance and carcass quality in intact male pigs. Livest. Prod. Sci. 92, 31-38.

Jia, X., Ekman, M., Grove, H., Færgestad, E.M., Aass, L., Hildrum, K.I. & Hollung, K., 2007. Proteome changes in bovine longissimus thoracis muscle during the early postmortem storage period. J. Proteome Res. 6, 2720-2731.

Jia, X., Hollung, K., Therkildsen, M., Hildrum, K.I. & Bendixen, E., 2006. Proteome analysis of early post-mortem changes in two bovine muscle types: M. longissimus dorsi and M. semitendinosis. Proteomics 6, 936-944.

Jia, X., Veiseth-Kent, E., Grove, H., Kuziora, P., Aass, L., Hildrum, K.I. & Hollung, K., 2009. Peroxiredoxin-6-a potential protein marker for meat tenderness in bovine longissimus thoracis muscle1. J. Anim. Sci. 87, 2391-2399.

Johnson, B.J. & Chung, K.Y., 2007. Alterations in the physiology of growth of cattle

with growth-enhancing compounds. Vet. Clin. N. Am. -Food A. 23, 321-332

Joseph, P., Nair, M.N. & Suman, S.P., 2015. Application of proteomics to characterize and improve color and oxidative stability of muscle foods. Food Res. Int. 76, 938-945.

Joseph, P., Suman, S.P., Rentfrow, G., Li, S. & Beach, C.M., 2012. Proteomics of muscle-specific beef color stability. J. Agr. Food Chem. 60, 3196-3203.

Karplus, P.A., 2015. A primer on peroxiredoxin biochemistry. Free Radical Bio. Med. 80, 183-190.

Kim, N.K., Cho, S., Lee, S.H., Park, H.R., Lee, C.S., Cho, Y.M., Choy, Y.H., Yoon, D., Im, S.K. & Park, E.W., 2008. Proteins in longissimus muscle of Korean native cattle and their relationship to meat quality. Meat Sci. 80, 1068-1073.

Klaavuniemi, T., Kelloniemi, A. & Ylänne, J., 2004. The ZASP-like motif in actinin-associated LIM protein is required for interaction with the α-actinin rod and for targeting to the muscle Z-line. J. Biol. Chem. 279, 26402-26410.

Kondoh, H., Lleonart, M.E., Bernard, D. & Gil, J., 2007. Protection from oxidative stress by enhanced glycolysis; a possible mechanism of cellular immortalization. Histol. Histopathol. 22, 85-90.

Kragh-Hansen, U., 1981. Molecular aspects of ligand binding to serum albumin. Pharmacol. Rev. 33, 17-53.

Krcmery, J., Camarata, T., Kulisz, A. & Simon, H.G., 2010. Nucleocytoplasmic functions of the PDZ-LIM protein family: new insights into organ development. BioEssays 32, 100-108.

Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H.J., Roepstorff, P. & Bendixen, E., 2003. Postmortem proteome changes of porcine muscle related to

tenderness. J. Agr. Food Chem. 51, 6992-6997.

Lametsch, R., Kristensen, L., Larsen, M.R., Therkildsen, M., Oksbjerg, N. & Ertbjerg, P. 2006. Changes in the muscle proteome after compensatory growth in pigs. J. Anim. Sci. 84, 918-924.

Lametsch, R., Roepstorff, P. & Bendixen, E., 2002. Identification of protein degradation during post-mortem storage of pig meat. J. Agr. Food Chem. 50, 5508-5512.

Lanferdini, E., Lovatto, P.A., Melchior, R., Orlando, U.A.D., Ceccantini, M. & Poleze, E., 2013. Feeding surgically castrated, entire male and immunocastrated pigs with different levels of amino acids and energy at constant protein to energy ratio with or without ractopamine. Livest. Sci. 151, 246-251.

Lean, I.J., Thompson, J.M. & Dunshea, F.R., 2014. A meta-analysis of zilpaterol and ractopamine effects on feedlot performance, carcass traits and shear strength of meat in cattle. PLOS ONE 9, e115904. doi: 10.1371/journal.pone.0115904

Lehninger, A.L., Nelson, D.L. & Cox., M.M., 2005. Lehninger principles of biochemistry (4th ed.). W.H. Freeman, New York, USA.

Li, W.J., Zhao, G.P., Chen, J.L., Zheng, M.Q. & Wen, J., 2009. Influence of dietary vitamin E supplementation on meat quality traits and gene expression related to lipid metabolism in the Beijing-you chicken. Brit. Poultry Sci. 50, 188-198.

Liu, C.Y. & Mills, S.E., 1990. Decreased insulin binding to porcine adipocytes in vitro by beta-adrenergic agonists. J. Anim. Sci. 68, 1603-1608.

Loneragan, G.H., Thomson, D.U. & Scott, H.M., 2014. Increased mortality in groups of cattle administered the β-Adrenergic agonists ractopamine hydrochloride and zilpaterol hydrochloride. PLOS ONE 9, e91177. doi:

10.1371/journal.pone.0091177

Lynch, A., Kerry, J.P., O'Sullivan, M.G., Lawlor, J.B.P., Buckley, D.J. & Morrissey, P.A., 2000. Distribution of α-tocopherol in beef muscles following dietary α-tocopheryl acetate supplementation. Meat Sci. 56, 211-214.

Lynch, M.P., Faustman, C., Chan, W.K.M., Kerry, J.P. & Buckley, D.J., 1998. A potential mechanism by which α-tocopherol maintains oxymyoglobin pigment through cytochrome b5 mediated reduction. Meat Sci. 50, 333-342.

Ma, X., Zheng, C., Hu, Y., Wang, L., Yang, X. & Jiang, Z., 2015. Dietary L-arginine supplementation affects the skeletal longissimus muscle proteome in finishing pigs. PLOS ONE 10, e0117294. doi: 10.1371/journal.pone.0117294

MacGillivray, A.J. & Rickwood, D., 1974. The heterogeneity of mouse-chromatin nonhistone proteins as evidenced by two-dimensional polyacrylamide-gel electrophoresis and ion-exchange chromatography. Eur. J. Biochem. 41, 181-190.

Maltin, C., Balcerzak, D., Tilley, R. & Delday, M., 2003. Determinants of meat quality: tenderness. P. Nutr. Soc. 62, 337-347.

Mancini, R.A. & Hunt M.C., 2005. Current research in meat color. Meat Sci. 71, 100-121.

McNeel, R.L. & Mersmann, H.J., 1999. Distribution and quantification of beta1-, beta2-, and beta3-adrenergic receptor subtype transcripts in porcine tissues. J. Anim. Sci. 77, 611-621.

Melody, J.L., Lonergan, S.M., Rowe, L.J., Huiatt, T.W., Mayes, M.S. & Huff-Lonergan, E., 2004. Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J. Anim. Sci. 82, 1195-1205.

Mercier, Y., Gatellier, P., Viau, M., Remignon, H. & Renerre, M., 1998. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. Meat Sci. 48, 301-318.

Mersmann, H.J., 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76, 160-172.

Mills, S.E., Spurlock, M.E. & Smith, D.J., 2003. β-adrenergic receptor subtypes that mediate ractopamine stimulation of lipolysis. J. Anim. Sci. 81, 662-668.

Mitsumoto, M., Arnold, R.N., Schaefer, D.M. & Cassens, R.G., 1993. Dietary versus postmortem supplementation of vitamin E on pigment and lipid stability in ground beef. J. Anim. Sci. 71, 1812-1816.

Montowska, M. & Pospiech, E., 2013. Species-specific expression of various proteins in meat tissue: proteomic analysis of raw and cooked meat and meat products made from beef, pork and selected poultry species. Food Chem. 136, 1461-1469.

Morgan, J.B., 1997. Implant program effects on USDA beef carcass quality grade traits and meat tenderness. Proc. Symp. Impact Implants Perform. Carcass Value Beef Cattle, Oklahoma State Univ., Stillwater, USA. pp. 147-154

Morzel, M., Chambon, C., Hamelin, M., Santé-Lhoutellier, V., Sayd, T. & Monin, G., 2004. Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. Meat Sci. 67, 689-696.

Nair, M.N., Costa-Lima, B.R.C., Schilling, M.W. & Suman, S.P., 2017. Proteomics of color in fresh muscle foods. In: From Paddock to Plate: Proteomics in Food Science. Ed: Colgrave, M., Elsevier, Oxford, United Kingdom. pp. 163 – 175

Nair, M.N., Suman, S.P., Chatli, M.K., Li, S., Joseph, P., Beach, C.M. & Rentfrow, G.,

2016. Proteome basis for intramuscular variation in color stability of beef semimembranosus. Meat Sci. 113, 9-16.

Needham, T. & Hoffman, L.C., 2015. Physical meat quality and chemical composition of the longissimus thoracis of entire and immunocastrated pigs fed varying dietary protein levels with and without ractopamine hydrochloride. Meat Sci. 110, 101-108.

Neethling N.E., Suman, S.P., Sigge, G.O., Hoffman, L.C. and Hunt, M.C., 2017. Exogenous and endogenous factors influencing color of fresh meat from ungulates. Meat Muscle Biol. (In press)

Niki, E. & Traber, M.G., 2012. A history of vitamin E. Ann. Nutr. Metab. 61, 207-212.

Niki, E., 2014. Role of vitamin E as a lipid-soluble peroxyl radical scavenger: in vitro and in vivo evidence. Free Radical Bio. Med. 66, 3-12.

Niki, E., Jyunichi, T., Yasuyuki, Y., Yorihiro, Y. & Yoshio, K., 1986. Oxidation of lipids. XIII. Antioxidant activities of α-, β-, γ-, and δ-tocopherols. B. Chem. Soc. Jpn. 59, 497-501.

NRC, 2000. Nutrient requirements of beef cattle (7th ed.). National Academies Press, Washington, D.C., USA.

O'Farrell, P.H., 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250, 4007-4021.

O'Sullivan, M.G., Kerry, J.P., Buckley, D.J., Lynch, P.B. & Morrissey, P.A., 1997. The distribution of dietary vitamin E in the muscles of the porcine carcass. Meat Sci. 45, 297-305.

Olivo, R., Scares, A.L., Ida, E.I. & Shimokomaki, M., 2001. Dietary vitamin E inhibits poultry PSE and improves meat functional properties. J. Food Biochem. 25,

271-283.

Ordway, G.A. & Garry, D.J., 2004. Myoglobin: an essential hemoprotein in striated muscle. J. Exp. Biol. 207, 3441-3446.

Patience, J.F., Rossoni-Serão, M.C. & Gutiérrez, N.A., 2015. A review of feed efficiency in swine: biology and application. J. Anim. Sci. Biotechno. 6, 33.

Perkins, A., Nelson, K.J., Parsonage, D., Poole, L.B. & Karplus, P.A., 2015. Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. Trends Biochem. Sci. 40, 435-445.

Perng, M.D., Cairns, L., IJssel, P.V.D., Prescott, A., Hutcheson, A.M. & Quinlan, R.A., 1999a. Intermediate filament interactions can be altered by HSP27 and alphaB-crystallin. J. Cell Sci. 112, 2099-2112.

Perng, M.D., Muchowski, P.J., IJssel, P.V.D., Wu, G.J.S., Hutcheson, A.M., Clark, J.I. & Quinlan, R.A., 1999b. The cardiomyopathy and lens cataract mutation in αB-crystallin alters its protein structure, chaperone activity, and interaction with intermediate filaments in vitro. J. Biol. Chem. 274, 33235-33243.

Peshavaria, M. & Day, I.N., 1991. Molecular structure of the human muscle-specific enolase gene (ENO3). Biochem. J. 275, 427-433.

Picard, B., Lefaucheur, L., Berri, C. & Duclos, M.J., 2002. Muscle fiber ontogenesis in farm animal species. Reprod. Nutr. Dev. 42, 415-431.

Pivovarova, A.V., Mikhailova, V.V., Chernik, I.S., Chebotareva, N.A., Levitsky, D.I. & Gusev, N.B., 2005. Effects of small heat shock proteins on the thermal denaturation and aggregation of F-actin. Biochem. Bioph. Res. Co. 331, 1548-1553.

Polati, R., Menini, M., Robotti, E., Millioni, R., Marengo, E., Novelli, E., Balzan, S. &

Cecconi, D., 2012. Proteomic changes involved in tenderization of bovine longissimus dorsi muscle during prolonged ageing. Food Chem. 135, 2052-2069.

Pollard, T.D. & Cooper, J.A., 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu. Rev. Biochem. 55, 987-1035.

Pomies, P., Macalma, T. & Beckerle, M.C., 1999. Purification and characterization of an alpha-actinin-binding PDZ-LIM protein that is up-regulated during muscle differentiation. J. Biol. Chem. 274, 29242-29250.

Pomies, P., Pashmforoush, M., Vegezzi, C., Chien, K.R., Auffray, C. & Beckerle, M.C., 2007. The cytoskeleton-associated PDZ-LIM protein, ALP, acts on serum response factor activity to regulate muscle differentiation. Mol. Biol. Cell 18, 1723-1733.

Pompeu, M.A., Rodrigues, L.A., Cavalcanti, L.F.L., Fontes, D.O. & Toral, F.L.B., 2017. A multivariate approach to determine the factors affecting response level of growth, carcass, and meat quality traits in finishing pigs fed ractopamine. J. Anim. Sci. 95, 1644-1659.

Ponsuksili, S., Murani, E., Phatsara, C., Schwerin, M., Schellander, K. & Wimmers, K., 2009. Porcine muscle sensory attributes associate with major changes in gene networks involving CAPZB, ANKRD1, and CTBP2. Funct. Integr. Genomic. 9, 455-471.

Poynton, R.A. & Hampton, M.B., 2014. Peroxiredoxins as biomarkers of oxidative stress. BBA-Gen. Subjects 1840, 906-912.

Preston, R.L., 1999. Hormone containing growth promoting implants in farmed livestock. Adv. Drug Deliver. Rev. 38, 123-138.

Pyle, W.G., Hart, M.C., Cooper, J.A., Sumandea, M.P., Tombe, P.P.D. & Solaro, R.J., 2002. Actin capping protein. Circ. Res. 90, 1299-1306.

Quinn, M.J, Reinhardt, C.D., Loe, E.R., Depenbusch, B.E., Corrigan, M.E., May, M.L. & Drouillard, J.S., 2008. The effects of ractopamine-hydrogen chloride (Optaflexx) on performance, carcass characteristics, and meat quality of finishing feedlot heifers. J. Anim. Sci. 86, 902-908.

Quinn, M.J., Walter, L.J., Swingle, R.S., Defoor, P.J., Harper, L.B. & Lawrence, T.E., 2016. Comparison of the effects of Actogain or Optaflexx on finishing feedlot steer performance and carcass characteristics. Prof. Ani. Sci. 32, 455-460.

Rabilloud, T. & Lelong, C., 2011. Two-dimensional gel electrophoresis in proteomics: a tutorial. J. Proteomics 74, 1829-1841.

Rabilloud, T., Chevallet, M., Luche, S. & Lelong, C., 2010. Two-dimensional gel electrophoresis in proteomics: past, present and future. J. Proteomics 73, 2064-2077.

Rathmann, R.J., Mehaffey, J.M., Baxa, T.J., Nichols, W.T., Yates, D.A., Hutcheson, J.P., Brooks, J.C., Johnson, B.J. & Miller, M.F., 2009. Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. J. Anim. Sci. 87, 3686-3701.

Rhee, S.G., Woo, H.A., Kil, I.S. & Bae, S.H., 2012. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. J. Biol. Chem. 287, 4403-4410.

Ricke, E.A., Smith, D.J., Feil, V.J., Larsen, G.L. & Caton, J.S., 1999. Effects of ractopamine HCl stereoisomers on growth, nitrogen retention, and carcass

composition in rats. J. Anim. Sci. 77, 701-707.

Rikard-Bell, C., Curtis, M.A., van Barneveld, R.J., Mullan, B.P., Edwards, A.C., Gannon, N.J., Henman, D.J., Hughes, P.E. & Dunshea, F.R., 2009. Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. J. Anim. Sci. 87, 3536-3543.

Rincker, P.J., Killefer, J., Matzat, P.D., Carr, S.N. & McKeith, F.K., 2009. The effect of ractopamine and intramuscular fat content on sensory attributes of pork from pigs of similar genetics. J. Muscle Foods 20, 79-88.

Roche, M., Rondeau, P., Singh, N.R., Tarnus, E. & Bourdon, E., 2008. The antioxidant properties of serum albumin. FEBS Lett. 582, 1783-1787.

Russell, B., Curtis, M.W., Koshman, Y.E. & Samarel, A.M., 2010. Mechanical stress-induced sarcomere assembly for cardiac muscle growth in length and width. J. Mol. Cell Cardiol. 48, 817-823.

Sayd, T., Morzel, M., Chambon, C., Franck, M., Figwer, P., Larzul, C., Roy, P.L., Monin, G., Chérel, P. & Laville, E., 2006. Proteome analysis of the sarcoplasmic fraction of pig semimembranosus muscle: implications on meat color development. J. Agr. Food Chem. 54, 2732-2737.

Schafer, D.A., Korshunova, Y.O., Schroer, T.A. & Cooper, J.A., 1994. Differential localization and sequence analysis of capping protein beta-subunit isoforms of vertebrates. J. Cell Biol. 127, 453-465.

Schiaffino, S. & Reggiani, C., 2011. Fiber types in mammalian skeletal muscles. Physiol. Rev. 91, 1447-1531.

Shi, X., Li, C., Cao, M., Xu, X., Zhou, G. & Xiong, Y.L., 2016. Comparative proteomic analysis of longissimus dorsi muscle in immuno- and surgically castrated male

pigs. Food Chem. 199, 885-892.

Shibata, M., Matsumoto, K., Oe, M., Ohnishi-Kameyama, M., Ojima, K., Nakajima, I., Muroya, S. & Chikuni, K., 2009. Differential expression of the skeletal muscle proteome in grazed cattle. J. Anim. Sci. 87, 2700-2708.

Smith, D.J., 1998. The pharmacokinetics, metabolism, and tissue residues of beta-adrenergic agonists in livestock. J. Anim. Sci. 76, 173-194.

Smith, G.C., Morgan, J.B., Sofos, J.N. & Tatum, J.D., 1996. Supplemental vitamin E in beef cattle diets to improve shelf-life of beef. Anim. Feed Sci. Tech. 59, 207-214.

Smith, S.B., Garcia, D.K. & Anderson, D.B., 1989. Elevation of a specific mRNA in longissimus muscle of steers fed ractopamine. J. Anim. Sci. 67, 3495-3502.

Spurlock, M.E., Cusumano, J.C., Ji, S.Q., Anderson, D.B., Smith, C.K., Hancock, D.L. & Mills, S.E., 1994. The effect of ractopamine on beta-adrenoceptor density and affinity in porcine adipose and skeletal muscle tissue. J. Anim. Sci. 72, 75-80.

Suman, S.P. & Joseph, P., 2013. Myoglobin chemistry and meat color. Annu. Rev. Food Sci. T. 4, 79-99.

Suman, S.P. & Nair, M.N., 2017. Current developments in fundamental and applied aspects of meat color. In: New Aspects of Meat Quality: From Genes to Ethics. Ed: Purslow, P.P., Elsevier, Oxford, United Kingdom, pp. 115-127.

Suman, S.P., Faustman, C., Stamer, S.L. & Liebler, D.C., 2007. Proteomics of lipid oxidation-induced oxidation of porcine and bovine oxymyoglobins. Proteomics 7, 628-640.

Suman, S.P., Hunt, M.C., Nair, M.N. & Rentfrow, G., 2014. Improving beef color stability: practical strategies and underlying mechanisms. Meat Sci. 98, 490-

504.

Suman, S.P., Rentfrow, G., Nair, M.N. & Joseph, P., 2014. Proteomics of muscle- and species-specificity in meat color stability. J. Anim. Sci. 92, 875-882.

Traber, M.G. & Atkinson, J., 2007. Vitamin E, antioxidant and nothing more. Free Radical Bio. Med. 43, 4-15.

Tracy, M.R. & Hedges, S.B., 2000. Evolutionary history of the enolase gene family. Gene 259, 129-138.

Vallenius, T., Scharm, B., Vesikansa, A., Luukko, K., Schäfer, R. & Mäkelä, T.P., 2004. The PDZ-LIM protein RIL modulates actin stress fiber turnover and enhances the association of α-actinin with F-actin. Exp. Cell Res. 293, 117-128.

Wang, L., Lei, M., Zuo, B., Xu, D., Ren, Z. & Xiong, Y., 2010. Multiple alternative splicing and differential expression of actinin-associated LIM protein (ALP) during porcine skeletal muscle development in vitro and in vivo. Meat Sci. 84, 655-661.

Wang, L., Xu, Y., Wang, Y., Zhong, T., Tang, G., Li, L., Zhang, H. & Xiong, Y., 2014. Identification and characterization of a differentially expressed protein (CAPZB) in skeletal muscle between Meishan and Large White pigs. Gene 544, 107-113.

Winterholler, S.J., Parsons, G.L., Reinhardt, C.D., Hutcheson, J.P., Nichols, W.T., Yates, D.A., Swingle, R.S. & Johnson, B.J., 2007. Response to ractopamine-hydrogen chloride is similar in yearling steers across days on feed. J. Anim. Sci. 85, 413-419.

Wood, Z.A., Schröder, E., Robin Harris, J. & Poole, L.B., 2003. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem. Sci. 28, 32-40.

Wu, J., Nair, M.N., Suman, S.P., Li, S., Luo, X., Beach, C.M., Bohrer, B.M. & Boler, D.D., 2017. Ractopamine-induced changes in sarcoplasmic proteome profile of post-rigor pork semimembranosus muscle. S. Afr. J. Anim. Sci. 47, 640-647.

Wu, W., Yu, Q.Q., Fu, Y., Tian, X.J., Jia, F., Li, X.M. & Dai, R.T., 2016. Towards muscle-specific meat color stability of Chinese Luxi yellow cattle: a proteomic insight into post-mortem storage. J. Proteomics 147, 108-118.

Xia, H., Winokur, S.T., Kuo, W.L., Altherr, M.R. & Bredt, D.S., 1997. Actinin-associated LIM protein: identification of a domain interaction between PDZ and spectrin-like repeat motifs. J. Cell Biol. 139, 507-515.

Xiong, Y.L., Gower, M.J., Li, C., Elmore, C.A., Cromwell, G.L. & Lindemann, M.D., 2006. Effect of dietary ractopamine on tenderness and postmortem protein degradation of pork muscle. Meat Sci. 73, 600-604.

Yamauchi, R., 1997. Vitamin E: mechanism of its antioxidant activity. Food Sci. Technol. Int. 3, 301-309

Yin, S., Faustman, C., Tatiyaborworntham, N., Ramanathan, R., Maheswarappa, N.B., Mancini, R.A., Joseph, P., Suman, S.P. & Sun, Q., 2011. Species-specific myoglobin oxidation. J. Agr. Food Chem. 59, 12198-12203.

Yoshida, Y., Niki, E. & Noguchi, N., 2003. Comparative study on the action of tocopherols and tocotrienols as antioxidant: chemical and physical effects. Chem. Phys. Lipids 123, 63-75.

VITA

Hyun Mok Kim
University of Kentucky
Department of Animal and Food Science

EDUCATION

2006-2012 Bachelor of Animal Science, Pusan National University, South Korea

PROFESSIONAL POSITIONS

2014-Present Graduate Student, Department of Animal and Food Sciences, University of Kentucky, Lexington, KY

2010-2012 Meat Processing Lab Assistant, Department of Animal Sciences, Pusan National University, South Korea

SCHOLASTIC AND PROFESSIONAL HONORS

2016 Initiated to Gamma Sigma Delta

PROFESSIONAL PUBLICATIONS

Kim H.M., Suman, S.P., Li, S., Nair, M.N., Beach, C.M., Edenburn, B.M., Boler, D.D., Dilger, A.C. & Felix, T.L., 2017. Ractopamine influences muscle proteome profile of postmortem beef longissimus lumborum. American Meat Science Association Reciprocal Meat Conference, College Station, TX, June 18-21. Abstract # 133.

Hyun Mok Kim